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Determination of methionine and lysine requirements of growing broilers using the Ideal Protein Concept

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DETERMINATION OF METHIONINE AND LYSINE REQUIREMENTS OF GROWING BROILERS USING THE IDEAL PROTEIN CONCEPT

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ABSTRACT

Three experiments were conducted to evaluate the responses of broiler performance to Lysine (Lys) and Methionine (Met) during the starter, grower, and finisher feeding phases. All the experimental diets were formulated based on the Ideal Protein Concept.

Experiment 1 evaluated the responses to Lys and Met on live performance of young broiler chickens from 0 to 18 d of age. The mean amino acid ratios to Lys suggested by literature values were used in the formulation based on the Ideal Protein Concept. Six levels of Lys and eight levels of supplemental Met were used in the diets resulting in a total of 48 experimental treatments. There were significant effects of Lys levels and added Met levels on feed intake (FI), body weight (BW) and feed conversion ratio (FCR) ($P \leq 0.05$). Significant interactions were also observed between Lys and added Met in response to these parameters ($P \leq 0.05$). There were differences in the estimated ratios of Met or total sulfur amino acids (TSAA) to Lys required for optimizing FI, BW, and FCR for chicks fed different Lys levels. These results indicated that the optimal ratios of indispensable amino acids to Lys may depend on dietary Lys level in the diet.

Experiment 2 evaluated the response to Lys and Met in diets on live performance of young broiler chickens during the grower period of 14-35 d. Experimental diets were designed similarly as the experiment 1 with six levels of Lys and eight levels of supplemental Met. Two consecutive trials using the same experimental diets were conducted with identical design. There were significant effects of dietary Lys levels on FI, BW and FCR ($P \leq 0.05$), with optimal Lys level for FI, BW and FCR of 1.20, 1.10 and 1.12, respectively. There were significant effects of added Met levels on BW and FCR ($P \leq 0.05$). No significant interactions between Lys and Met were observed based on FI, BW and FCR. There were differences in the estimated ratios of Met or TSAA to Lys required for optimizing FI, BW, and FCR for broiler chickens fed different Lys levels. Results of this study suggest that the response to variation in Lys level is independent of Met level and optimal ratio of Met or TSAA to Lys varies with different dietary Lys level in the diets.

Experiment 3 was conducted to evaluate the response to Lys and Met in diets on live performance of broiler chickens during the finisher period of 35-49 d. Similar design as the previous two experiments was used

with six levels of Lys and four levels of supplemental Met. There were significant effects of dietary Lys levels on body weight gain (BWG) and FCR, with optimal Lys level for BWG and FCR of 1.01 and 1.05, respectively. There was a significant effect of supplemental Met on FCR. No significant interactions were observed between Lys and supplemental Met for FI, BWG, and FCR. Increasing Lys level significantly improved dressing percentage and breast meat yield. There were differences in the estimated ratios of Met or TSAA to Lys required for optimizing FI, BWG, and FCR for broiler chickens fed different Lys levels. Results of this study suggest that the response to variation in Lys level is independent of Met level in broiler finisher diets and that the ideal amino acid profile may depend on the Lys level in the diets.

In conclusion, the ideal ratios of Met and TSAA to Lys based on the Ideal Protein Concept vary for broiler chickens fed different Lys level in the diets at each of the growing phases.

This dissertation is approved for recommendation to the Graduate Council

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DEDICATION

I dedicate this dissertation to all of my family members and those who helped me in my life.

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Part I LITERATURE REVIEW

1. Basis for Dietary Protein and Amino Acids

Proteins are made of strings of amino acids that form chains known as peptides. While there are hundreds of amino acids in nature, only twenty two different amino acids are commonly found in proteins. The primary structure of proteins is the sequential order in which the amino acids are covalently linked, as well as, the location of disulfide bond which may be present. The primary structure of a protein determines its three-dimensional structure, which in turn determines its properties. The secondary structure of a protein is the arrangement in space of the backbone, which is stabilized by hydrogen bonds. Some of the most important backbone arrangements are α-helix, β- pleated sheet, and β-turn. They can be combined in a number of ways to produce structural motifs of many proteins. The tertiary structure of proteins includes the three-dimensional arrangement of all the atoms in the protein. The primary structure of a protein contains all the information needed to specify the tertiary structure. The quaternary structure of proteins is the arrangement of subunits in multi-subunit proteins (Campbell and Farrell, 2006).

Much of the dry matter fed to chickens is comprised of protein. Since proteins have been shown to be composed of individual amino acids, the dietary crude protein (CP) requirement for monogastric animals is the requirement of each of the amino acids *per se*. From the information of protein synthesis, there are twenty-two amino acids in body proteins and all of them are physiologically essential to the animal (Bedford and Summers, 1985). Nutritionally, these amino acids can be divided into two categories: 1) amino acids that cannot be synthesized by poultry at all or not rapidly enough to meet metabolic requirements are called essential or indispensable amino acids; 2) those that can be synthesized by poultry have been termed non-essential or dispensable amino acids. The essential amino acids (EAA) must be supplied by the diet. The nonessential amino acids (NEAA) must be synthesized by poultry if they are not supplied by the diet (NRC, 1994). The EAA needed by all species of poultry include methionine (Met), lysine (Lys), threonine (Thr), tryptophan (Trp), histidine (His), isoleucine (Ile), leucine (Leu), arginine (Arg), valine (Val), and phenylalanine(Phe) (Donald el al., 2002). The distinction between EAA and NEAA may, under some circumstances, depend on the concentration of other amino acids in the diet and the response variable used to determine the degree of adequacy. Thus, there is a third classification of amino acids termed as conditional EAA that includes tyrosine (Tyr) and cysteine (Cys).

Requirements for these amino acids, under specific conditions, can be met partially by dietary supplements (Leeson and Summers, 2001). Tyrosine essentiality depends directly on the presence of phenylalanine since tyrosine is synthesized from phenylalanine. Similarly, the essentiality of cysteine is dependent upon the presence of methionine since cysteine has been shown to be synthesized by methionine (D' Mello, 1994). Some amino acids that birds can synthesize but cannot produce enough for optimum growth are considered as semi-essential amino acids. Those amino acids include glycine (Gly), serine (Ser), and proline (Pro) (Donald el al., 2002). Compared with mammals, poultry have a higher requirement for glycine because it forms a component of the uric acid molecule.

All animal species commonly synthesize and degrade proteins. During the process of breaking down proteins, amino acids can be reused for protein synthesis; meanwhile the non-essential amino acids can be produced. Approximately 80% of these amino acids from the protein degradation, under normal conditions, can be reutilized for synthesis of protein or other amino acids (Millward *et al*, 1975).

The National Research Council (NRC) stated that broiler chickens do not have specific requirements for CP *per se*; nevertheless, an adequate amount of CP is necessary to provide adequate nitrogen for the synthesis of non-essential amino acids. Chickens fed low CP diets with adequate EAA supplements had a reduced growth performance when compared to the chickens fed a diet with more dietary CP containing adequate EAA (Huyghebaert and Pack, 1996; Kerr and Kidd, 1999). In birds, the majority of the intact protein consumed eventually is taken in as peptides. Therefore, it is possible that the need for a minimum amount of dietary CP is the result of the fact protein absorption is mainly taken as peptides (Rerat, 1992). Dietary CP can provide non-essential amino acids; therefore there is an additional need for non-essential nitrogen when broiler chicks are fed a starter diet reduced by 4% CP (Corzo *et al*, 2005).

2. The Ideal Protein Concept

Several things shape the ideal protein concept: 1) increasing concerns about environmental impacts such as nitrogen and phosphorus pollution resulting from animal production; 2) available sophisticated feed formulation programs; 3) and the consideration of economical production (Emmert and Baker, 1997). Because of the differences in physiological, environmental, dietary, and genetic factors, the amino acid requirements of broiler chicks vary considerably. It is almost impossible to determine the accurate individual amino acid requirement under various conditions (Baker *et al,* 2002; Schutte and de Jong, 1999). Research was conducted to determine a single reference amino acid under various conditions in order to easily estimate the requirements of other EAA provided that the proportions of other EAA to the reference amino acid are unaffected under various conditions and stages. The ideal protein concept refers to a blend of EAA that meet the requirement for protein accretion and maintenance in an animal with no deficiencies and no excesses (Emmert and Baker, 1997). The advantage of applying the concept of an ideal protein is that the requirements of other EAA can be easily determined under various conditions once the reference amino acid requirement is accurately established under a specific circumstance. The ideal protein concept has been using lysine as the reference amino acid even though methionine has been shown to be the primary limiting amino acid. Lysine was selected as the reference amino acid for the following reasons: 1) lysine is the second limiting amino acid in most commercial poultry diets and the supplementation is economically available; 2) the analysis of lysine is a relatively simple procedure; 3) dietary lysine is utilized only for protein accretion and maintenance of broiler chicks; 4) there are a number of research projects conducted to determine the lysine requirements of different broiler strains under various conditions (Emmert and Baker, 1997).

Birds cannot totally digest amino acids in most ingredients except in their synthetic form. The digestibility of most EAA from various sources for broiler chicks is different. The ideal protein concept could eliminate the differences in absorption and utilization of various amino acid sources if the ideal ratios are based upon the digestible levels of dietary amino acids. Use of an ideal protein is recommended in a corn soybean meal diet even though the digestibility values among EAA in this particular diet are similar (Emmert and Baker, 1997). Nonetheless, the NRC (1994) reported the amino acid requirements for broiler chicks based on total amino acids instead of providing digestible ideal amino acid ratios of individual essential amino acid to lysine. Dietary lysine requirement is different when various response variables, such as body weight gain (BWG), feed conversion ratio (FCR), and breast meat yield (BMY), are selected. There is a hierarchy of lysine requirements depending upon the response variable selected: BWG < BMY < FCR < minimum abdominal fat (Leclercq, 1998). Several other authors have shown that the dietary lysine requirement for optimal breast meat yield is higher than that needed for optimal live performance traits, such as BWG (Kerr *et al*, 1999; Gorman and Balnave, 1995; Kidd, *et al*, 1998). In contrast, the estimation of the requirements of tryptophan, threonine, isoleucine, and valine for optimal BWG and FCR have been shown to be almost identical (Baker *et al*, 2002). Leclerq (1998) confirmed that the threonine requirement is the same when either FCR, BWG, BMY, or abdominal fat was chosen as the response variable. In addition to the response variable, Leclerq (1998) showed that the type of model being used to estimate the amino acid requirement can also affect the ideal amino acid requirement profile. In his study, it showed that the ratio of threonine to lysine, or the ratio of valine to lysine for optimal performance is higher if the "broken line" model is used than if the monomolecular model is used. Broiler strain may also have an effect on the lysine requirement and ideal amino acid profile. Leclerq *et al* (1994) reported that genetically lean birds showed a lower growth rate than that of the genetically fat birds fed a diet deficient in either lysine or arginine. Alleman *et al* (1999) suggested that the requirement of threonine for genetically lean male broiler chicks is higher than that for genetically fat male broiler chicks when the quadratic polynomial equation was used. This may be due to a lower daily feed intake of threonine in the lean broiler strain. Leclercq and Beaumont (2001) reported that genetically lean broiler chicks require a higher lysine concentration in the diet than genetically fat broiler chicks. However, Han and Baker (1991) showed that the lysine requirement was almost the same for both fast-growing (Hubbard x Hubbard) and slow-growing (New Hampshire x Columbian) broiler chickens. They explained that the daily feed consumption of the fast-growing strain was over twice as much as that of the slow-growing strain. Alleman *et al* (1999) thus suggested that feed intake or appetite should account for differences of essential amino acid requirements between genotypes. However, Gous and Morris (1985) pointed out several disadvantages of this method: the amino acid profile changed in different treatment diets, which affects the responses of broiler chickens; the range of the amino acid levels was limited by the practical formulation of diets; plus, some amino acids are costly to use to make this range. Therefore, Gous and Morris (1985) suggested the diet dilution method could overcome the above disadvantages. This method is based on sequential dilution using a protein free mixture diet added to a summit diet in which all the EAA largely exceed the dietary requirements except the one under evaluation, which is significantly deficient in the diet (Fisher and Morris, 1970). Table 1 shows the comparison of ideal ratios for amino acids reported in the literature.

3. Methods Used for Determination of Amino Acid Requirements of Broilers

Numerous research studies have been conducted to determine the essential amino acid requirements. However, it is not easy to obtain accurate dietary amino acid needs for poultry, which is due partially to the following reasons: the growth response to the change of dietary amino acid levels is not linear; antagonism or toxicity may occurr between or among amino acids; anti-nutritive factors or interactions between some amino acids and other nutrients; the metabolizable variation under physiological and environmental conditions(Oviedo-Rondon and Waldroup, 2002). Two common methodologies are empirical and factorial assessments. Empirical evaluation is based upon the overall response of an animal to an increasing level of the nutrient in the diet; while the factorial method is based on the calculated amino acid requirement to a specific response variable such as lean tissue growth or breast meat yield.

The most common empirical method being used to determine a single amino acid requirement in broiler chickens is using graded levels of a supplemental amino acid added to a basal diet that is deficient in that particular amino acid (Gous and Morris, 1985; Mack *et al*, 1999). The basal diet usually contains about 80% of the 1994 NRC specification of the amino acid being determined. It is important that the level of the amino acid being measured in the basal diet should be deficient enough to be growth limiting. Also, the range of the levels of a particular amino acid supplemented should be wide enough to obtain the complete responses (Gous, 1986).

Another method to estimate the need of a specific amino acid in terms of a targeted response is to separate the requirements into the maintenance requirement and performance requirement (Owens and Pettigrew, 1989). This method can improve the efficiency of the nitrogen accretion. Growth modeling technique is used to predict the daily amino acid requirements, which seems to be more accurate and profitable than the use of fixed requirements for the three phases of broiler chickens: starter, grower, and finisher. The indicator amino acid oxidation method was used by Ewing et al, (2001) to determine the amino acid requirement of broiler chicks in their early age. It is suggested that this technique is useful to determine the amino acid requirements of broiler chickens at specific ages.

4. Dietary Lysine Requirement in Broiler Chickens

Lysine, as a second limiting AA in corn and soybean meal based diet, has been used worldwide for more than twenty-five years. There are numerous studies described in the literature covering the lysine requirements for broiler chickens under various circumstances. Lysine requirements depend on various factors such as age, sex, strain, ambient temperature, as well as level of ME and CP.

4.1 Effect of Age on Lysine Requirement

Lysine requirement expressed as a percentage of the diet tends to decrease as birds age (NRC 1994). However, the lysine requirements are different in terms of the measured production parameters such as BWG, FCR, and breast meat yield. For the first 3 wks, it appears that the lysine requirement for optimal FCR is higher than that for BWG (Vazquez and Pesti, 1997; Hurwitz *et al.*, 1998; Knowles and Southern, 1998; Garcia and Batal*,* 2005; Zaghari *et al*, 2002; Garcia *et al,* 2006). Whereas, Ando *et al,* (1989) reported that the optimal dietary lysine level for BWG was higher than that of FCR for the first 3 wks with 0.82% and 0.81%, respectively. Usama *et al.* (2007) also suggested that lysine requirements for BWG and feed efficiency during 4-21 days were found to be 0.98% and 0.97%, respectively. Leclercq (1998) suggested that there was a hierarchy of lysine requirements according to the response criteria used with BWG < BMY < FCR < minimum AF. There are trials reporting that breast meat yield is responsive to lysine level above that required for BWG (Kerr et al, 1999; Han and Baker, 1994; Dozier et al, 2008). Garcia et al (2006), however, stated that during the grower stage (21 to 38 d), the dietary Lys requirement for maximum breast meat yield was similar to the estimation for maximal BWG. Ng'ambi et al (2008) suggested that the dietary lysine to CP ratio for optimum breast meat yield was lower than that for optimum growth rate and FCR.

4.2 Effect of Sex on Lysine Requirement

Generally, the lysine requirement for male broiler chickens is higher than that of female broiler chickens (Thomas *et al.*, 1977; Han and Baker, 1993; Han and Baker, 1994; Zaghari et al, 2002; Garcia et al, 2006; Dozier et al, 2008; Dozier *et al.*, 2009).It is often recognized that male broilers contain more protein and less fat in their carcass composition than those in female broilers (Twining *et al.*, 1978;Han and Baker, 1991; Han and Baker, 1993; Han and Baker, 1994; Zaghari *et al.*, 2002), and therefore male broilers require higher amino acid levels than females, whereas, when FCR was selected as the response parameter, the lysine requirements for both male and female broilers are very close with 0.97% and 1.00% digestible lysine(dLys), respectively from 7 to 21 days and 0.96% dLys for both male and female from 21 to 38 days (Garcia et al, 2006), but Han and Baker (1994) stated that lysine requirement for FCR of male broilers is higher than that of female broilers during 21 to 42 days with 0.85% and 0.80%, respectively.

4.3 Effect of Strain on Lysine Requirement

The lysine requirement for broiler chickens is influenced by the genetic difference (Han and Baker, 1993; Edwards *et al.*, 1999; Ajayi and Ejiofor, 2009). Lean and efficient broilers required diets with a higher lysine level (Leclercq and Beaumont, 2001). However, Han and Baker (1991) stated that lysine requirement expressed as a percentage of the diet was almost the same for both fast-growing (Hubbard x Hubbard) and slow-growing (New Hampshire x Columbian) broiler chickens in terms of BWG and feed efficiency, the fast-growing strain basically consumed about twice as much as the slow-growing strain consumed daily, and the digestible lysine requirement for optimal FCR was 675 mg/day for fast-growing broiler chickens compared with 318 mg/day for slow-growing broiler chickens. The fast-growing strain chickens consumed more feed daily than the slow-growing chickens but the portion of protein composition of the body gain was approximately the same in both strains. This demonstrated that the absolute value of lysine requirement is higher for fast-growing strain broilers than that for slow-growing broiler chickens.

4.4 Effect of Dietary CP Level on Lysine Requirement

The dietary lysine requirement is influenced by the dietary CP level in the diet. It was found that as the protein level increased, the lysine requirement for maximum BW increased at a particular protein level, whether the lysine requirement was expressed as percentage of the diet or as the weight of lysine consumed per day per unit of body weight (Grau, 1948). The lysine requirement levels apparently decreased as dietary protein levels decreased from 23% and 25% to 18% and 20%, respectively, based on BWG and FCR (Hurwitz *et al.*, 1998). The lysine requirement of broiler chickens for BWG and FCR to the first three wks of age increased as dietary CP was increased from 170 to 250 g CP/kg diet, but no difference of lysine requirement was found when CP level was higher than 250 g/kg diet (Maria, 2003). Plumstead *et al.* (2007) proposed the similar result that the digestible lysine requirement of broilers was dependent upon the dietary CP levels; the BWG of broilers during the first 3 wks increased and continued responding to digestible lysine above 1.19% when CP and AA concentrations increased. The lysine requirement of the chicks during the starter period was a linear function of dietary CP content rather than a fixed percentage of the diet provided the protein levels ranged between 14 and 28% (poultry research report 11). Since the lysine level was influenced by various CP levels, there might be a ratio of lysine level to CP level. Plumstead (2005) stated that the BWG of 21 d old broilers responded in a linear manner to increasing lysine levels, which maintained with the same ratio of 5.5% to CP levels increased from 21.8% to 26.9%. Plumstead *et al.* (2007) further stated that when a fixed ratio of digestible lysine to CP was applied and other essential AAs were not limiting, the BWG and FCR of 20 d old broilers responded positively to increasing digestible lysine up to at least 1.32% (27.2% CP). Similar results were also reported by Urdaneta-Rincón and Leeson (2008), supporting the idea that when the ratio of lysine to CP is kept constant at 5.7%, maximal productive performance of broiler chickens during the first 18 days can be attained among 21 and 25% CP, but a significant lower carcass fat content was seen in the diet with 25% CP. The most efficient scenario of lysine and CP levels based on muscle protein deposition in broiler chicks of 21 d of age was the diet with 210g/kg CP and 1.22% lysine level (Maria, 2003).

4.5 Effect of Metabolizable Energy Level on Lysine Requirement

Broilers possess a good ability to control their energy intake at a relatively constant level (Boomgaardt and Baker, 1973; Leeson *et al.*, 1996). Energy levels in the diets influence feed intake of broilers, except the first several days post-hatch (Molenaar *et al.*, 2009). In general, feed intake was reduced as dietary energy level increased (Fisher and Wilson, 1974; Waldroup *et al.*, 1976a; Harris *et al,* 1977; Noy and Sklan, 2002; Saleh *et al.*, 2004), therefore other nutrients in the diet should be kept in balance with changes in energy. When energy increases or decreases the amount of other nutrients will increase or decrease in proportion. The BWG, water content, and protein content reached optimal status at ratios of lysine to nitrogen-corrected true metabolizable energy (TMEn) of between 0.76 and 0.86 g per megajoule (MJ) in broiler chickens before 43 days (Sibbald and Wolynetz, 1990).

There was no interaction between the metabolisable energy (ME) levels and lysine levels, whereas the carcass yield and breast meat weight increased with higher levels of energy and lysine in the diets (Araujo *et al.*, 2005). A similar result was found by Plumstead *et al.* (2007) who found no interaction of ME and digestible lysine on BWG or FCR. Increasing the ME from 3000 to 3200 kcal/kg had no effect on feed intake, which may be due to a fixed ratio of digestible lysine to CP and essential AAs that were maintained in graded increments of CP. Mabray and Waldroup (1981) stated that when the whole carcass weight was measured there was a significant interaction between AA levels and energy levels, within each of the EAA levels of 70, 80, 90, 100, 110 and 120% of the NRC requirements (1977). The whole carcass weight increased with increasing energy concentration of the diet, whereas the abdominal fat pad weight increased as the energy value increased in the diet. The abdominal fat pad weight decreased with the increasing levels of AAs and there was a significant interaction between energy value and AA levels with respect to abdominal fat pad weight. Regarding this, a high level of lysine is needed to reduce the abdominal fat pad weight. Based on the performance, tissue deposition and the multivariate canonical variable, a metabolizable lysine level of 1.18% with ME level at 3,000 kcal/kg for 21 d of broilers is desired when the broken line model was used and a metabolizable lysine level of 1.22 % with ME level at 3,100 or 3,200 kcal/kg when the linear model was used (Bellaver *el al.,* 2002).

4.6 Effect of Ambient Temperature on Lysine Requirement

The temperature in the chicken house affects production performance. As the house temperature decreases the chicken will eat more feed to keep warm, and the chicken will eat less to keep cool as the house temperature increases. High ambient temperature will adversely affect feed intake and BWG. Those reports are well documented (Waldroup, 1982; Leeson, 1986; Mendes *et al.*, 1997; Alleman and Leclercq, 1997; Cheng *et al.*, 1997b; Han and Baker, 1993). Increasing relative humidity under high environmental temperatures also decreases BWG and feed intake (Harris *et al.*, 1977). Reduced performance during hot weather is one of the most severe problems in the poultry industry. Because of reduced feed intake under hot temperature, it seems logical and common to adjust the CP or AA levels of the diet. However, Adams *et al.* (1962) conducted a trial of increased CP levels to alleviate the adverse effect of increasing ambient temperatures ranging from 21°C to 32 °C on the performance of broiler chickens from 4 to 8 wk of age. The results showed that increasing the CP levels failed to attenuate the adverse effect of increasing environmental temperature. A similar finding was suggested by Cowan and Michie (1978). On the other hand, decreasing CP level also did not seem a good way to alleviate the adverse effect on broiler chickens under high environmental temperature (Alleman and Leclercq, 1997). Han and Baker (1993) found that heat stress of 37°C reduced BWG and feed intake of 22 d old broiler chickens by about 22%, and the lysine requirement increased for females, but the lysine requirement did not increase for male broilers. The influence of environmental temperature on the lysine requirement of broiler chickens was also investigated by McNaughton *et al.* (1978). Two to four wk broiler chickens in two trials fed different dietary lysine levels were maintained at 15.6 °C and 29.4 °C temperatures. The lysine requirement did not increase under the high environmental temperature. The optimal BWG and FCR were obtained at the dietary lysine level of 1.10% in the 15.6°C environment, the dietary lysine requirements for optimal BWG and FCR in the 29.4 °C environmental temperature was 1.00% and 0.95%, respectively, which actually indicated a decreased dietary lysine requirement as the environmental temperature increased. Waldroup *et al.* (1976b) proposed that this might be because the excess amino acids in the diet have more adverse effects under heat stress conditions upon the feed intake and BWG. Therefore the diets might be formulated to have minimal excess of amino acids so that the performance cannot be impaired or even be improved under heat stress. Influence of dietary lysine levels on performance of broilers under heat (25.5 to 33.3°C) or cold stress (15.5°C) during the period of 3 to 6 wks of age was conducted by Mendes *et al.* (1997). The results showed that there were no significant effects of lysine levels on BWG, feed intake, FCR, mortality, or dressing percentage. On the other hand, the leg quarter yield and abdominal fat content were significantly affected by lysine levels with decreased abdominal fat content as the lysine level increased. The breast meat yield improved as the lysine level increased under cold environmental temperatures. These similar responses were further confirmed in the same lab (Ojano–Dirain and Waldroup, 2002). The lysine requirement for maximum performance and protein deposition in the carcass of 22 to 42 d of broiler chickens kept under 25.6°C of environmental temperature is 1.05% of total lysine in the diet (Borges *et al.*, 2002). Corzo *et al.* (2003) suggested that there was no significant effect of increased dietary lysine levels on the performance of 6 to 8 wks of age male broilers except FCR, which was linearly improved as the dietary lysine level increased. The overall dietary lysine requirement for this period was no less than 0.95%, which actually was higher than the previously obtained level that was under similar conditions without heat stress. This might be because of the reduced feed intake. This reduced feed intake needs to be accommodated by increasing the dietary lysine concentration. However under heat stress the absolute lysine need seems decreased.

4.7 Effect of Arginine Antagonism on Lysine Requirement

The antagonism between lysine and arginine is a complex amino acid interaction that has been well established and extensively investigated in poultry since the 1950's (Jones, 1964; O'Dell and Savage, 1966; Jones *et al.*, 1967; D'Mello and Lewis, 1970; Kadirvel *et al.*, 1974; Kadirvel and Kratzer, 1974; Austic and Scott,1975; Chamruspollert *et al.*, 2002; Balnave and Brake, 2002;). Excess dietary lysine impairs arginine absorption by decreasing the availability of arginine, and thus induces an arginine deficiency and increases the apparent requirement for arginine. This antagonism can happen especially in diets composed of casein or gelatin or a combination of both since both casein and gelatin are rich in lysine and poor in arginine (Jones, 1964). A possible mechanism for the antagonism might be that both lysine and arginine compete for absorption from the digestive tract based upon the fact that both of them are essential amino acids. Nevertheless, Jones *et al.* (1967) reported that excess L-lysine did not interfere with the digestion or absorption of arginine when monitoring the concentration of two enzymes, trypsin and carboxy-peptidease B, of which excess lysine is an in vitro inhibitor. The comparable maximal increases in postprandial plasma arginine concentration between the lysine containing ration and the control diet further demonstrated that lysine affects arginine utilization not by altering digestion or absorption of arginine, but by increasing the kidney arginase activity, which results in the loss of arginine. Further experiments in the same lab showed that the increased kidney arginase activity occurred several days later than the decreased plasma arginine level occurred, which indicated that the initial decreased plasma arginine level was not due to the hydrolysis of arginine by arginase. They finally postulated that the primary effect of lysine was to increase catabolism of arginine within the body or to decrease the arginne level via competition between lysine and arginine at the renal tubular absorptive surface. Kadirvel *et al.*(1974) employed the everted sac technique to measure the uptake of lysine and arginine by the small intestine and further concluded that excess lysine induced arginine deficiency was not at the absorption level and may be due to a metabolic effect. It is likely to be recognized that a reduction in the reabsorption of arginine from renal tubules accounts for the antagonism between lysine and arginine when lysine was infused into the vena of cockerels (Boorman *et al.*, 1968). Dietary excess lysine has more effect on increasing kidney arginase activity in a strain of high arginine requirement chicks than a strain of chicks with low arginine requirement (Nesheim, 1968). An interaction between arginine, lysine and glycine in the diet of chickens was reported and the presence of glycine alleviated the growth depression by excess lysine in the diet that contains plant proteins (Kadirvel *et al.*, 1974).The growth stimulating effect of excess glycine in the diet with suboptimal arginine level was also reported by O'Dell and Savage (1966). In the same experiment, it was also shown that the effect of excess arginine in a diet with low lysine (lysine/arginine = 0.5) was not as dramatic as in the case of excess lysine in the diet with low arginine. When the diet was adequate in terms of lysine, dietary excess arginine was not detrimental. This was further confirmed by the evidence for a true metabolic antagonism between arginine and lysine (O'Dell and Savage, 1966). There is an interaction existing between electrolytes and dietary Arg: Lys ratio, which was first investigated by Jones (1961). He reported that excessive dietary lysine in the casein-gelatine based diet decreased the potassium concentration and increased the sodium concentration of muscle. A similar effect of excessive dietary lysine on cellular potassium and sodium concentration was also observed in his subsequent studies (Jones, 1964) when the same basal diet was used. Since excess dietary lysine in a casein based diet decreases the potassium concentration, O'Dell and Savage (1966) evaluated the effect of dietary potassium acetate supplementation on the lysine and arginine antagonism induced diets in which protein was from casein (30%), soybean (26%) or sesame (30%) meal. The results showed that potassium acetate supplementation in the casein basal diet produced a significant stimulation of growth with or without added lysine. Similarly, Stutz *et al.* (1971) noted that the addition of either potassium and sodium salts containing metabolizable anions or arginine-HCl or a combination of both, with cation to anion ratio of 1:1, produced notable growth stimulation. They found that the growth rate stimulated by cation supplement was about the same as the response to arginine (0.6%) supplement alone and this cation supplement can decrease the typical arginine deficiency symptoms such as ataxia and frizzled feathers. Moreover, by supplementing either potassium acetate or arginine·HCl, the concentration of free lysine in plasma or muscle of broilers fed casein based diet was decreased and arginine levels were increased in plasma and muscle. When both of them were supplemented in the casein based diet, the effect on plasma and muscle lysine levels was more significant and similar to those observed in broilers fed a practical corn-soybean meal diet. These results conclude that cation supplementation can mitigate the lysine and arginine antagonism and spare the increasing arginine requirement in casein-based diets. This effect might be because better cation balance decreases the rate of arginine catabolism. Dietary excessive anions such as chloride may exacerbate the lysine-arginine antagonism, which was demonstrated by Calvert and Austic (1981). When the concentration of dietary chloride (provided by calcium chloride) increased from 4.4 to 18.4 g/kg in corn gluten meal based diets, there was no significant effect on plasma lysine or arginine concentration of the growing chickens. In addition, BWG was decreased and FCR was increased with a higher concentration of dietary chloride. This effect was more dramatic in those chickens fed diets with higher lysine level. This adverse effect of excessive chloride can be alleviated by adding higher levels of dietary arginine to the diet containing a high lysine level. Because of this antagonism, a basal diet with low arginine and high lysine levels will need supplementation of dietary arginine or cation. The ratio of Arg to Lys in casein is about 0.5. This low ratio in casein no doubt requires increased arginine supplementation if the chicks were fed casein as their source of amino acids. Broiler chickens fed for 4 wks with diets containing a ratio of Arg: Lys around 1.0 showed maximal growth rate (O'Dell and Savage, 1966). Similarly, experiments conducted by Labadan *et al.* (2001) also indicated that the requirement patterns of lysine and arginine were similar for broiler chickens through 8 wks of age, except for the starting stage in which the lysine requirement appeared to be slightly higher than that of arginine. Data from various classical studies indicated that the optimum ratio of Arg: Lys ranges from 0.8 to 1.7 depending upon different circumstances such as dietary electrolyte balance or during heat stress (Balnave and Brake, 2002). Under thermoneutral and high temperatures, Brake *et al.* (1998) conducted four experiments with diets containing different dietary Arg: Lys ratios of 0.94, 1.05, 1.08, 1.09, 1.10, 1.20, 1.25, 1.34, 1.36, 1.37, 1.39, and 1.49 for broiler chickens during the period of 20 d of age until 56 d of age. The results showed that under high temperatures, increasing the Arg: Lys ratio produced consistent improvement in FCR without jeopardizing the growth rate. The optimum Arg: Lys ratio needed for FCR was reduced at high temperatures when increased concentration of dietary sodium chloride was added to these diets. When the uptake of arginine by intestinal epithelium of broilers was measured, the level was significantly reduced under heat stress compared with those of broilers at thermoneutral temperatures when an equimolar concentration of lysine was present. Their subsequent studies (Balnave and Brake, 1999) showed that at high temperature (31°C), increasing dietary arginine: lysine ratio through 1.05, 1.15, 1.25, to 1.35 consistently improved feed intake and BWG with 3-6 wk old broilers. Moreover, the supplementation of sodium bicarbonate to these diets caused a greater increase in feed intake and BWG with the increasing ratio of Arg: Lys at 31°C. Mendes *et al.* (1997) also reported that the improved FCR was observed with increasing Arg: Lys ratios from 1.1 to 1.4 in the diets for 21 to 42 d old broiler chickens exposed to heat or cold stress, whereas the improved weight gain was not observed. These results indicated that increased optimum ratio of dietary Arg: Lys or supplementation of electrolyte was needed to maintain optimal performance of broilers under heat or cold stress.

Balnave *et al.* (1999) reported that methionine activity sources affected optimum arginine: lysine ratio due to the effect of feed intake. The addition of equimolar DL-methionine (DLM) or 2-hydroxy-4-(methylthio) butanoic acid (HMB) affected the optimum dietary Arg:Lys ratio in diets for broilers under heat stress. The increasing supplement of HMB from 0.16 to 0.32% in the diet with Arg: Lys ratio of 1.36 gave a significant increase in BWG due to better feed intake in the period of 21 to 38 days at 32 °C, whereas increasing DLM in the same diets did not have these effects. In another trial, the broilers fed HMB with the diets containing Arg: Lys ratios of 1.03, 1.20, or 1.34 consistently produced better BWG and FCR with a higher feed intake from 42 to 48 days compared to those broilers fed diets with DLM. The broilers fed the DLM added diet with an Arg:Lys ratio of 1.03 had the best growth response of all. In agreement with these results, Balnave and Brake (2002) found that under heat stress feed intake seems to be the primary response of the broilers fed diets supplemented with different dietary methionine activity sources and concluded that the optimum dietary Arg:Lys ratio for broilers under heat stress depends on the dietary methionine activity source.

Waldroup *et al.* (2006a) demonstrated that differences in diet type may influence the response to Arg and Lys. The first dietary set was formulated with corn-soybean meal and corn gluten meal (CGM) supplemented with L-lysine HCl and L-Arg to provide four Lys levels of 1.1, 1.2, 1.3, and 1.4% as well as five Arg levels of 1.25, 1.35, 1.45, 1.55, and 1.65%. The second dietary set was formulated independently using corn-soybean meal and CGM to provide the same four Lys and five Arg levels. Broiler chickens from day-old to 21 days of age were fed these two sets of diets. The results showed there were no significant differences in BW or FCR for the first set of diets, whereas there were significant interactions in BW and FCR for Arg and Lys in the second set of diets. This might be because high level of CGM reduced feed intake and therefore reduced performance (Waldroup *et al.*, 2002). These results indicated that differences in diet type might influence the optimum Arg: Lys ratio for the performance of broiler chickens due to the formulated ingredients known to have reduced digestibility, amino acid imbalance, poor palatability, or various anti-nutritive factors, but not amino acid levels *per se*.

Since excess dietary Lys can increase the chick's Arg requirement and excess Arg can increase the chick's methionine (Met) requirement, dietary Lys and Met might be also interrelated. Chamruspollert *et al.* (2002a) conducted a study to evaluate the dietary interrelationships among Arg, Met, and Lys in young broiler chicks of 1 to 14 d of age. The results showed that there was Arg and Met interaction in which Arg toxicity was dependent on the Met level of the diets for broilers, plus, a three-way interaction among Arg, Met, and Lys was observed based upon BWG, feed intake, and muscle creatine. These results might indicate that the interaction between Arg and Met also influence the Lys requirement.

5. Dietary Methionine or Total Sulfur Amino Acids (TSAA) Requirement in Broiler Chickens

Methionine and cystine are the only two amino acids that contain sulfur. These two sulfur-containing amino acids, especially methionine, are very important in that these are required by poultry for a number of functions and are primary deficient in soybean meal. The major functions of methionine are as an essential component for protein synthesis, as a methyl donor and as a precursor of cysteine. When cysteine is in deficiency and methionine is in excess in the diet, the physiological requirement of cysteine can probably be met by the conversion of methionine to cysteine (Graber and Baker, 1971). However, the conversion of cysteine to methionine did not occur when Rose and Rice (1939) conducted an investigation with rats, whereas it has been demonstrated that cystine can replace part of the dietary requirement of methionine (Finkelstein *et al.*, 1988; White and Beach, 1937; Sasse and Baker, 1974). Because of the interrelationship between Met and Cys, the requirements of Met and Cys, as shown in table 2, are also stated as TSAA levels. The requirement of Met for broilers of different ages falls somewhere between 0.30% and 0.71% based upon various criteria such as FCR and BWG. The estimation of TSAA during different stages ranges from 0.72% to 1.12% based on desired criteria.

5.1 Effect of Age on Methionine or TSAA Requirement

As shown in table 2, the requirement of Met or TSAA basically decreases as broilers age. However, Waldroup and Hellwig (1995) reported that the Met and TSAA requirements for maximum rate of egg production, egg weight and daily egg mass did not reduce with age and production stage for the egg-type hens. Instead, it showed increased requirements during the middle and last quarter of egg production and that peak daily requirement for Met was similar with 384 mg/day for egg production, 380 mg/day for egg weight and 402 mg/day for egg mass.

5.2 Effect of Sex on Methionine or TSAA Requirement

Bornstein and Lipstein (1966) conducted four trials to determine the TSAA requirement of broilers during the finishing period from 5 to 10 wks of age. The results showed that no difference of TSAA requirements was observed between male and female broilers in terms of maximal BWG and optimal FCR. Lumpkins *et al.* (2007) proposed that during the starter period for broilers of 7 to 19 d of age, the average digestible sulfur amino acid (DSAA) requirements for males and females were similar based upon FCR and BWG. During the grower period from 21 to 42 d of age, the DSAA requirement of FCR for males was higher (0.64%) than for females (0.57%). The estimated DSAA requirement of maximal BWG for males was similar to females with the level of 0.55%. The DSAA level for BMY of male and female is 0.55% and 0.56%, respectively, which is very similar to the BWG requirement. The requirement of carcass yield for male and female is 0.51% and 0.52%, respectively. All of these levels of DSAA, based on various criteria, are similar and indicate that the effect of sex on the variation of DSAA requirements is little and should not be considered in feed formulation. The data shown in table 2 also indicates the similar requirements of DSAA in terms of sex.

5.3 Effect of strain on Methionine or TSAA Requirement

Moran (1994) conducted an investigation on the response of two broiler strains, Ross x Arbor Acres (RxAA) and Steggles x Arbor (SxAA), to inadequate Met based on live performance and processing yields. Both strains were fed diets adequate and deficient in Met during 0 to 3 wks (0.65% vs. 0.42%), 3 to 6 wks (0.54% vs. 0.46%) and 6 to 8 wks (0.35% vs. 0.30%). The levels of cystine in all of these diets exceeded NRC (1984) recommendation. The results showed that RxAA broilers overall had higher live weights than SxAA, but SxAA had better FCR. The RxAA birds had more abdominal fat when processed at both 6 and 8 wks than SxAA birds due to a higher feed intake of RxAA birds. Both strains had similar chilled carcass yield (without abdominal fat) but both decreased as a result of low methionine during these stages. The BMY was reduced in both strains fed low methionine but SxAA had even lower BMY than RxAA birds. The different responses of these two strains to abdominal fat and feed intake may indicate the difference in Met requirements for different strains. Nevertheless, Kalinowski *et al.* (2003a) demonstrated that there was no significant difference between strain and Met requirement when the experiments were conducted with fast- (Ross × 3F8) and slow- (Ross × 308) feathering male broilers. Both strains were fed diets containing adequate dietary Cys of 0.50% with 0.35, 0.40, 0.45, and 0.50% Met. The results showed that FCR improved, with optimal Met level of 0.50%, as Met level increased without significant difference between these two feathering types from 0 to 3 wk of age. There was no significant difference of nitrogen retention between feathering types when measured from 20 to 21 d of age with optimal Met at 0.46%; however, other experimental diets containing suboptimal Met level of 0.45% with various Cys levels indicated that the estimated Cys requirement for fast-feathering (0.44%) males was higher than for slow-feathering males (0.39%). A subsequent study from the same lab with the same strains (Kalinowski *et al.*, 2003b) suggested that the overall Met requirement for male broilers between 3 and 6 wk of age was approximately 0.46% and further demonstrated that there was no significant effect of feathering strains on the Met requirement. The estimated Cys requirement for fast-feathering (0.42%) males was higher than for slow-feathering males (0.37%).

5.4 Effect of Dietary CP Level on Methionine or TSAA Requirement

Harms and Waldroup (1963) conducted experiments with laying hens fed low protein diets. The results indicated that when the layer diets contained 13%, 17% or 21% protein, Met becomes the first limiting amino acid. When the protein level is reduced below 13%, Met may become the second limiting amino acid. The supplementation of both methionine hydroxyl analogue calcium (MHAC) and Lys to the diet containing 11% protein improved the FCR. The addition of MHAC to the diet containing 13% protein improved the egg production rate and FCR. These results suggested the need for Met supplementation when diets contain a low protein level. Boomgaardt and Baker (1973) suggested that the TSAA requirement for young chicks stayed constant at about 4% of the CP (17.4%) at all three energy levels of 2,600, 3,000 and 3,400 kcal ME/kg. The TSAA requirement for maximum BWG, estimated by the method of least squares, was 3.97, 4.08 and 3.85% of the protein at 2,600, 3,000 and 3,400 kcal ME/kg diet, respectively; The estimated TSAA requirement for optimal FCR was 3.85, 4.02 and 3.51% of the protein at 2,600, 3,000 and 3,400 kcal ME/kg diet, respectively; The estimated TSAA requirement for maximizing protein retention was 3.97, 4.02 and 3.85% of the protein at 2,600, 3,000 and 3,400 kcal ME/kg, respectively. Vieira *et al.* (2004) further illustrated that the dietary TSAA requirement depends on dietary protein level and thus the TSAA requirement should be expressed in terms of protein level. Male Cobb 500 and Ross 308 broilers were both fed diets containing two dietary protein levels of 20.5% and 26.0% as well as four levels of digestible TSAA from 14 to 35 d of age. The results showed that 0.76% of true fecal digestible sulfur amino acid (TFD SAA) at adequate dietary protein level of 20.5% or 1.06% TFD SAA with the high protein diet of 26.0% was recommended for the Ross 308 broilers based on weight gain; about 0.85% TFD SAA at 20.5% protein or 1.15% TFD SAA at 26% protein was recommended based on FCR. Similarly, different TFD SAA level was also required by Cobb 500 at the two protein levels based on various performance criteria. However, Si *et al.* (2004) reported that there was no significant interaction between Met and protein levels when male broiler chicks were fed diets with 16, 18, 20, or 22% CP with 100 and 110% of NRC recommendations. There was no significant effect of Met exceeding 100% or 110% of NRC recommendation on improving the depressed performance of broilers fed diets containing 100 or 110% of NRC recommended levels of dietary essential amino acids and low in CP.

5.5 Effect of Metabolizable Energy Level on Methionine or TSAA Requirement

As broilers maintain a good ability to control their energy intake at a relatively constant level (Boomgaardt and Baker, 1973; Leeson *et al.*, 1996), other nutrients in the diet should be kept in balance with changes in energy level. It is likely that the Met or TSAA should be kept in proportion to energy content.

Boomgaardt and Baker (1973) reported that there was no significant interaction between energy level and TSAA level for young chicks from 8 to 21 d of age based upon BWG and FCR. Five levels of TSAA were fed at 1.72, 2.41, 3.10, 3.79 and 4.48% of the 17.4% CP. Each TSAA level consisted of 58% L-cystine and 42% DL-methionine. Three energy levels of 2,600, 3,000 and 3,400 kcal ME/kg were fed at each level of TSAA. The non-significant interaction between energy and TSAA level based on BWG and FCR was observed. However, the results also showed that there was a significant effect of increasing dietary energy level from 2,600 to 3,400 kcal ME/kg on body protein concentration and body fat concentration; as dietary energy level increased, the body protein concentration decreased, whereas the body fat concentration increased significantly. The supplementation of TSAA significantly increased body protein content and significantly decreased body fat content.

5.6 Effect of Rearing Environment on Methionine or TSAA Requirement

Feed intake is decreased in order to maintain homeothermy when broilers are raised under heat stress. It seems logical that diets should be fortified with protein, amino acids and other nutrients to counterbalance the reduced intake. However, there is still controversy as to increase or decrease amino acid levels and protein level under heat stress conditions (Gonzalez-Esquerra and Leeson, 2006). Moreover, the reduced feed intake cannot fully explain the depressed growth performance of broilers raised under high temperatures. Even with the same amount of feed intake, chicks gained less weight at 32°C than those raised at 22°C, with 55% less in young chickens (2 to 4 wks of age) and 22% less in older chickens (4 to 6 wks of age); therefore, heat stress resulted in decreased feed efficiency (Geraert *et al.,* 1996). Cheng *et al.* (1997a) reported that broilers raised under heat-stress (from 21.1 to 35°C) during 3 to 6 wks of age should not be fed diets with CP greater than 20% and 100% NRC recommended amino acid levels with 3250 kcal/kg ME, because it was observed that broilers under heat stress were highly sensitive to dietary CP and amino acid levels. They also found that supplementing 16 or 18% CP with methionine, lysine, threonine, tryptophan, and arginine to provide more than 100% NRC recommended amino acid levels in corn-soybean diets produced deleterious effects on FCR and body fat deposition at temperatures above 29.4°C. However, according to Ojano-Dirain and Waldroup (2002),broilers reared under constant moderate heat stress (26.7 °C) from 3 to 6 wk of age fed corn soybean meal diets containing 3250 kcal/kg ME and 20.67% CP require 0.44% methionine for optimal BWG, FCR, dressing percentage, breast meat yield and a numerical (P=0.08) reduction in abdominal fat. They suggested that under moderate heat stress, the Met requirement for maximum live performance or breast meat yield is higher than the NRC (1994) recommended level of 0.38%. Lumpkins *et al.* (2007) reported that male and female broilers reared on floor pens from 7 to 19 d of age required higher DSAA level of average 0.68% for optimal FCR than those birds reared in battery cages, which required 0.63% on average. However, the estimated DSAA requirements for maximal BWG of male and female broilers were very similar when reared either in battery or on floor pens.

5.7 Effect of Choline, Betaine and Copper on Methionine or TSAA Requirement

Derilo and Balnave (1980) reported that low dietary choline concentration reduced BWG and feed efficiency of broiler chickens fed semi-purified diets. These adverse effects were exacerbated by low dietary TSAA concentration. At low dietary choline concentrations, supplementation of methionine thus spared part of the dietary requirement of choline; on the other hand, high dietary concentration of choline (>1750 mg/kg) also increased the dietary TSAA requirement because increasing the dietary choline level with inadequate TSAA level may decrease the performance of broilers. These results indicated that increased TSAA level would be needed if the diets contained inadequate or excessive dietary choline concentration. Besides Methionine, choline and betaine are also methyl donors, so it is likely that choline and betaine might have Met-sparing effects**.** Waldroup *et al.* (2006b) conducted a study to evaluate this Met-sparing effect of choline and betaine for male broilers from 1 to 56 d of age based on BWG, parts yield, dressing percentage, FCR and tensile strength of intestinal segments. The results, however, showed that there was no apparent sparing effect of choline or betaine supplementation at 1,000 mg/kg or a combination of 500 mg/kg of both on Met needs, but choline or betaine improved feed efficiency at 35 and 42 d. In addition, there was similar positive effect of choline and betaine on breast yield which was independent of Met levels. No effect of choline or betaine on intestinal strength was observed in this study in which coccidiosis was not an apparent problem. Jensen *et al.* (1989) suggested that there was a significant interaction between copper and methionine in the diets. The presence of copper in the methionine supplemented diets improved feed efficiency more than those diets without copper for male and female broilers of 3 to 6 wk of age. Abdominal fat was significantly reduced by both methionine and copper supplemented diets for female, but not for male broilers. However, the absence of copper in methionine added diets improved BWG in male broilers more than in male broilers fed diets with copper. These results may conclude that the supplementation of copper in the Met added diets has positive effect on feed efficiency but has negative effect on BWG. Whereas, Waldroup *et al.* (1979) suggested that there was no significant interaction between copper and methionine levels when two trials were conducted with diets supplemented with copper sulfate at normal use levels up to 250 mg/kg, but a significant influence of copper levels on efficiency of feed utilization was observed in one of the two trials. They concluded that copper sulfate at levels up to 250 mg/kg did not significantly influence the sulfur amino acid requirement.

5.8 The Efficiency of Utilization of Methionine and its Analogues.

The D-, L-, DL-methionine and methionine analogues can all be utilized by poultry. The efficiency of utilizing various sources of Met might be different. Katz and Baker (1975) conducted assays with young male chicks fed diets containing D-Met or L- Met to evaluate the relative efficacy of D- and L-Met. The results indicated that at levels of supplementation close to the Met requirement, D- Met and L-Met had equal efficacy, however the L-Met supported faster and more efficient BWG than D-Met fed at levels below the Met requirement. When used as the sole source of sulfur amino acid, the requirement of L-Met or D-Met as estimated by least squares for maximum BWG was 0.58% and 0.59%, respectively. In the presence of 0.27% L-cystine, the requirements of L-and D- Met were estimated at 0.27% and 0.30%, respectively. In the same lab (Dilger and Baker, 2007), they also reported that there were no differences in growth performance of chicks from 8 to 20 d of age due to supplementation of L-Met vs. DL-Met. Cystine supplementation at 0.2% improved feed efficiency in that added L-cystine depressed feed intake by 6.9%, but BWG was reduced only by 3.6%. From these results, it may be concluded that there is no evidence to support the differences in effectiveness between L-Met and DL-Met in purified or practical-type low-protein diets containing various sulfur amino acid (SAA) levels. The bio-efficacy of liquid methionine is generally lower than that of dry methionine, but if the level of TSAA is set to the commercial recommendation, the bioefficacy of liquid methionine seems to be equal to that of dry methionine based on equimolar methionine (Bunchasak, 2009).

Waldroup and Whelchel (1983) conducted a study to evaluate the bio-effectiveness of liquid DL-methionine sodium salt compared with DL- Met in corn-soybean meal type diets. The results demonstrated that the bio-efficacy of liquid DL- Met sodium salt was fully equal to DL-Met based on equimolar levels of supplemented Met and thus concluded that the sodium salt of liquid Met is an effective Met supplementation resource for broiler chickens. In the same lab (Waldroup *et al.*, 1981), they also evaluated the bio-efficacy of another liquid Met supplement known as methionine hydroxyl analogue free acid (MHA-FA), and the broilers' performance was compared with that obtained when similar diets were supplemented with equal molars of L-Met, DL-Met, or the calcium salt of methionine hydroxyl analogue. The results showed the performance of chicks fed diets with MHA-FA was equal to those obtained from the diets containing other Met products; therefore, it may be concluded that MHA-FA could be used as an effective Met supplementation resource with the advantages of more even distribution in the diet, easier handling and lower cost of manufacturing. Lemme *et al.* (2002) conducted two experiments to assess the relative bio-efficacy of MHA-FA and DL-Met (DLM) for broilers of 1 to 42 d of age based on BWG, FCR, and carcass responses to dietary Met sources. Regression analysis indicated that liquid MHA-FA was 68% of BWG, 67% of feed conversion, 62% of carcass yield, and 64% of breast meat yield as efficacious as pure DLM on an as-fed basis, whereas responses to liquid MHA-FA and diluted DLM were very similar at corresponding supplementation levels. The results of the second trial showed that liquid MHA-FA was 72% of BWG, 51% of feed conversion, 48% of carcass yield, and 60% of breast yield as efficacious as DLM on a weight-for-weight basis. It was concluded that the average relative effectiveness of liquid MHA-FA was 62% compared to DLM. In agreement with this conclusion, Payne *et al.* (2006) conducted three experiments to assess the bioavailability of MHA-FA relative to DLM in broilers and concluded that the average bio-efficacy of liquid MHA-FA relative to DLM is 57% on a product basis based on BWG, FCR, and breast meat yield.

Featherston and Rogler (1978) suggested that there was antagonism of cystine on methionine utilization when the dietary level of methionine is suboptimal. When similar suboptimal levels of L-cystine were added to either crystalline amino acid or wheat-peanut meal diets containing 0.2% methionine, chick growth was depressed. When 0.4% methionine was added to the diets, the growth depressing effect of cystine was not observed. Recently, Baker (2009) reported that small excesses of cysteine depressed the growth of chicks fed methionine-deficient diets. In addition, a high level of dietary L-cysteine (2.5% or higher) is lethal and causes acute metabolic acidosis for young chicks. Moreover, high ratios of cysteine to methionine impair utilization of the hydroxy analog of methionine, but not of methionine itself. Motl *et al.* (2005b) conducted a study to determine if dietary Na levels influenced the utilization of DL-Met and the liquid form of 2-hydroxy-4-methylbutanoic acid (HMB) in diets for male broilers of 1 to 21 d of age. The Na levels in the diets were 0.15, 0.20, and 0.25% with a constant Cl level of 0.20%, and the levels of Met in the diets ranged from 0.33 to 0.61% in increments of 0.04%. The results showed that there was a significant interaction between Na level and Met level for both BW and FCR. Higher levels of dietary Na in the diets with lower levels of Met supplementation had adverse effects on both BW and FCR, independent of the two sources of Met. There was no difference of utilizing equimolar amounts of dry DL-Met (98% activity) or liquid HMB (88% activity) for broilers based on BW, feed conversion, or mortality. Using these two sources of Met, these authors conducted another study to assess the effects of intestinal modification by antibiotics and antibacterials on utilization of methionine sources by broiler chickens from 0 to 21 d of age. The intestinal modification was achieved by adding to the diets with a mixture providing 200 g/ton of bacitracin methylene disalicylate, 200 g/ton of chlortetracycline, 100 g/ton of penicillin, and 100 g/ton of sulfaquinoxaline. The results showed that there was no apparent effect of the feed additives on the utilization of these two sources of Met based on BWG, FCR, or mortality. Again, no difference, based on BWG, FCR, or mortality, was observed between broilers fed diets supplemented with equimolar amounts of dry DL-Met (98% activity) or HMB (88% activity).

Fatufe and Rodehutscord (2005) conducted a study to evaluate the effect of CP level on the efficiency of Met utilization in the diets for broilers of 8 to 21 d of age. They found that the marginal efficiency of Met utilization was, at its maximum, 8% lower with a normal CP level (22.9%) than with a low CP level (18.3%), and concluded that the efficiency of Met utilization was affected by the NEAA nitrogen concentration in the diet.

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Table 1 Comparison of ideal ratios for amino acids reported in the literature

Recommendations for amino acid ratios to lysine by various researchers																			
*AA	**1						2	3	4	5	6	7	8	9	10		ALL AGES		
	1-21		22-42		43-56			1-21	20-40	7-28	8-21	0-21	0-42	1-21	10-21	32-43			
	Dig	Tot	Dig	Tot	Dig	Tot	Dig	Dig	Dig	Dig	Dig	Dig	Dig	Tot	Dig	Dig	MEAN	LO	HI
Lys	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Arg	105	102	105	102	105	102	110	105	112	108		96	105	105	97	101	104	96	112
Gly								65									65		
Gly+Ser		150		140		135								150			144		
His	36	36	36	36	36	36		32		38		24		29	29	31	33	24	38
Ile	65	66	67	68	67	68	70	67	71	63	61	65	66	67	64	62	66	61	71
Leu	108	108	109	109	109	109		109		108		92		100	94	117	106	92	117
Met	39	39	40	40	40	40				37		38	38	42	35	36	39	35	42
TSAA	71	71	72	72	72	72	75	72	75	70		72	73	75	71	69	72	69	75
Phe	63	63	63	63	63	63				62				60			63		
Phe+Tyr	115	114	115	114	115	114		105		121				112	107	102	112	102	121
Pro								44									44		
Thr	65	68	65	68	65	68	65	67	63	66	56	62	65	67	63	65	65	62	68
Trp	16	16	17	17	17	17	18	16	19	14	17	18	16	17	16	18	17	14	19
Val	75	76	77	78	77	78	80	77	81	81	78	69	80	75	67	75	76	67	81

*AA- amino acid; Dig-digestible; Tot-total; LO-low; HI-high

**1. Rostagno *et al.*, 2005; 2. Schutte and de Jong, 1999; 3. Baker and Han, 1994; 4. Mack *et al.*, 1999; 5. Roth *et al.*, 2001; 6. Baker et al., 2002; 7. Austic, 1994; 8. Dutch Bureau of Livestock Feeding, 1996. 9. NRC 1994 with Lys adjusted to 1.20% and Gly+Ser at 1.80%; 10. Coon, 2004.

Table 2 Review of estimates of Met and TSAA requirements of broilers at different ages.

(Values in bold italic are digestible estimates).

Age(d)	Sex	Met estimate	TSAA estimate	Criteria	Authors
7-19	M		***0.71%***	FCR	Lumpkins, *et al.*, 2007
	F		***0.71%***	FCR	
	M		***0.67%***	BWG	
	F		***0.67%***	BWG	
	M/F		***0.68%***	FCR	
	M/F		***0.61%***	BWG	
21-42	M/F		***0.55%***	BWG	
	M/F		***0.56%***	BM Yield	
0-21	M		***0.82%***	BWG	Garcia and Batal, 2005
			0.82%	FCR	
21-42	M	0.44%		BWG/FCR	Ojano–Dirain and Waldroup, 2002
7-14	M	0.52%		FCR	Chamruspollert *et al.*, 2002b
	F	0.45%		FCR	
	M	0.54%		BWG	
	F	0.48%		BWG	
22-42	M		0.90%	Overall response	Rodrigueiro *et al.*, 2000
	F		0.86%	Overall response	
43-56	M		0.76%	Overall response	
	F		0.74%	Overall response	
14-34	M/F		0.95%	Maximum profit/FCR	Pack and Schutte, 1995
14-38	M/F		0.85%	FCR	
	M/F		0.89%	FCR/BM yield	
14-34/38	M/F		≥0.88%	Performance and carcass yield	Schutte and Pack, 1995
8-18	F	0.30%	0.66%	BWG	Ohta and Ishibashi,1994
4-14	F		1.14%	BWG	Koide *et al.*, 1993
14-24	F		1.11%	BWG	
4-24	F		1.12%	BWG	

24-34	F		1.03%	BWG	
34-44	F		0.99%	BWG	
24-44	F		1.00%	BWG	
4-14	F		1.18%	FCR	
14-24	F		1.08%	FCR	
4-24	F		1.10%	FCR	
24-34	F		1.05%	FCR	
34-44	F		1.02%	FCR	
24-44	F		1.03%	FCR	
4-14	F		0.97%	BWG	
21-31	F		0.89%	BWG	
4-14	F		0.97%	FCR	
21-31	F		0.93%	FCR	
21-42	M/F		0.78%	Overall response	Jensen *et al.*, 1989
1-21	M		0.87%	BWG	Oliveira Neto *et al.*,2005
	M		0.89%	FCR	
0-21	M/F	0.55%	0.88%	BWG	Waldroup *et al.*, 1979
	M/F	0.57%	0.90%	FCR	
28-56	M		0.50%	BWG	Adams *et al.*, 1962
28-56	M		0.65%	FCR	

Part II RESEARCH STUDIES

Chapter 1 Ratios of Methionine and Total Sulfur Amino Acids to Lysine in Broiler Starter Diets

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ABSTRACT A study was conducted to evaluate the separate response to Lysine (Lys) and Methionine (Met) in diets on live performance of young broiler chickensfrom 0 to 18 d of age. Corn and soybean meal of known protein and moisture content were used to formulate basal diets to provide 0.90 to 1.40% digestible Lys (dLys) in increments of 0.10%. The mean amino acid ratios to Lys suggested by literature values were used in the formulation based on the Ideal Protein Concept. All amino acids other than Met and TSAA were calculated to meet or exceed the expected ratios to Lys. Diets were calculated to be isocaloric with 3086 kcal/kg ME and were supplemented with inorganic trace mineral premix to avoid any source of Met from this premix. Experimental diets were prepared by adding variable amounts of MHA® (84% of Met) and cornstarch to the Lys basal diets to provide increments of 0.04% up to 0.28% supplemental Met activity for each level of digestible Lys, with a 6 × 8 factorial arrangement of 6 levels of Lys and 8 levels of supplemental Met resulting in a total of 48 treatments. Each of the 48 experimental diets was fed to six replicate pens of six male chicks (Cobb 500). Body weights by pen were obtained at 1 and 18 d of age with feed consumption determined during the test period. There were significant effects of dLys levels and added Met levels on feed intake (FI), body weight (BW) and feed conversion ratio (FCR) ($P \le 0.05$). Significant interactions were also observed between Lys and added Met in response to these parameters ($P \le 0.05$). There were differences in the estimated ratios of Met or TSAA to Lys required for optimizing FI, BW, and FCR for chicks fed different Lys levels. Therefore, the optimal ratios of indispensable amino acids to Lys may depend on dietary Lys level in the diet.

INTRODUCTION

It has become increasingly popular to formulate diets using the Ideal Protein Concept based on the following: 1) increasing concerns about environmental impacts such as nitrogen and phosphorus pollution resulting from animal production; 2) available sophisticated feed formulation programs; 3) and the consideration of economical production (Emmert and Baker, 1997). The ideal protein concept refers to a blend of essential amino acids that meet the requirement for protein accretion and maintenance of an animal with no deficiencies and minimal excesses using lysine as a reference amino acid (Emmert and Baker, 1997). Numerous reports have suggested various ratios of amino acids to lysine. Recommended ratios for methionine range from 35 to 42 per 100 units of lysine with a mean of 39, with TSAA ranging from 69 to 75 with a mean of 72. There is disagreement in the literature regarding the requirement for lysine. Also, there have been limited investigations related to the interaction and relationship between Lys and Met. Si *et al.* (2004) reported that there were no significant interactions between Lys and Met for BW, FCR, mortality, and processing parameters when both were fed equal to or in excess of NRC recommendations. Café and Waldroup (2006) conducted a study to evaluate the interactions between levels of Met and Lys in broiler diets based on feeding stages from current industry applications. The result showed no interactions for any performance parameters except for FCR and leg quarter yield at 42 d of age. In the formulation using the ideal protein concept, when Met and TSAA are held in a ratio to Lys, the concentration of these amino acids increase or decrease as Lys is increased or decreased. Since Met and TSAA are considered the primary limiting amino acids in corn-soybean meal diets, the response to variation in Lys in such situations may in fact be a response to these amino acids instead. Therefore, the objective of this study was to evaluate the separate response to Lys and to Met in diets for young broiler chickens 0 to 18 d of age.

MATERIALS AND METHODS

Dietary treatments

Corn and soybean meal of known protein and moisture content were used in formulating the diets. Amino acid values suggested by Degussa (Degussa AG Feed Additives, 2006), adjusted for the crude protein content of the diet, and amino acid digestion coefficients suggested by Heartland Lysine (Heartland Lysine, 1995) were assigned to the ingredients. The mean amino acid ratios to Lys suggested by literature values (Table 1) were used in formulation. Diets were formulated to provide 0.90 to 1.4% digestible Lys (dLys) in increments of 0.10%, resulting in six lysine basal diets. All amino acids other than Met and TSAA were calculated to meet or exceed the expected ratio to Lys. Other nutrients were formulated in the basal diets with the suggested values from Rostagno *et al.* (2005). Defluorinated phosphate was the primary source of supplemental phosphate because of its known beneficial effect on pelleting. All diets were calculated to be isocaloric with 3086 ME kcal/kg and were supplemented with complete vitamin and trace mineral premixes obtained from commercial sources. An inorganic trace mineral mix was used so as not to provide any source of methionine from this source. Table 2 shows the composition and calculated nutrient content of the basal diets with different levels of dLys formulated to average ideal ratios of other amino acids, except methionine, and total sulfur amino acids. Table 3 shows the calculated ratio of digestible amino acids to lysine in the basal diets.

Experimental diets were prepared by the addition of variable amounts of MHA (84% dry product) and cornstarch to the lysine basal diets. MHA was added to provide increments of 0.04% up to 0.28% supplemental Met activity for each of six lysine basal diets. The combination of the six levels of Lys and eight levels of added Met resulted in 48 dietary treatments. Following mixing, diets were steam pelleted in a California Pellet Mill Laboratory Model using a 3/32" (2.38 mm) die.

Birds and management

All procedures used during this study were approved by the University of Arkansas Animal Care Committee. Male chicks of a commercial broiler strain (Cobb 500) were obtained from a local hatchery where they had been vaccinated in ovo for Marek's disease and had received vaccinations for Newcastle Disease and Infectious Bronchitis post hatch via a coarse spray. Six chicks were randomly assigned to each of 288 compartments in electrically heated battery brooders with wire floors. Six replicate pens were assigned to each dietary treatment. The experimental diets and tap water were provided for ad libitum consumption. Supplemental feeders and waterers were used during the first seven days. Temperature and airflow were controlled by automatic heaters and ventilation fans. Fluorescent lights provided 24 hr of light daily. Care and management of the birds followed recommended guidelines (FASS, 2010).

Measurements

Samples of the basal diet within each level of dLys were analyzed for crude protein and total amino acid content by a commercial amino acid supplier. Samples of the diet within each level of dLys with the highest amount of supplemental Met were analyzed for content of supplemental amino acids. Body weights by pen were obtained at one and 18 d of age with feed consumption determined during the test period. Birds were checked twice daily and any bird that died or was removed to alleviate suffering was weighed with the weight used to adjust feed conversion.

Statistical analysis

Pen means served as the experimental unit for statistical analysis. Mortality data were transformed to $\sqrt{n+1}$. Data were presented as natural numbers. All statements of significance are based on $P \leq 0.05$. The data were subjected to factorial analysis with Lys level and added Met level as main effects along with the interaction between these two effects. The GLM procedure of SAS (SAS Institute, 1991) was used for the analysis. If there were significant differences among or between means of treatments, these means were separated using repeated *t*-tests based on probabilities generated by the LS means option. Nonlinear regression analysis was conducted to estimate the level of Met, TSAA, and ratios of Met:Lys and TSAA:Lys that provide the greatest response in FI, BW and FCR at each level of digestible lysine, using the PROC NLIN procedure of SAS (SAS Institute, 1991).

RESULTS AND DISCUSSION

Analyzed crude protein and amino acid contents in the basal diets were in close agreement with the calculated values, except the first basal diet with 0.9% dLys level. The analyzed MHA activity felt within calculated values (Table 4).

Table 5 shows the effects of various levels of dLys and added Met on feed intake, body weight and feed conversion ratio. There was a significant effect of dLys level on feed intake, body weight and feed conversion ratio ($P \leq 0.05$). Increasing the dLys levels up to 1.2% resulted in significant improvement of feed intake. Further increasing Lys levels reduced feed intake. The effect of dLys levels on BW is similar to the effect on FI with decrease of BW as dLys level increased from 1.2% to 1.4%. The FCR was reduced in a linear manner as dLys levels increased to 1.4%. The effects of increasing Lys levels are in agreement with Kidd *et al.* (1997), who reported that increasing dietary lysine level from 1.10 to 1.20% improved BW and FCR of 1 to 18d old chicks. For the responses of BW and FCR to Lys levels, the results here were in agreement with Han and Baker (1993) and Baker *et al.* (2002) in that the dietary Lys requirement for optimal FCR was higher than that for maximal BW gain for broiler chicks. Garcia et al. (2006) conducted a study to evaluate the variations of digestible lysine requirement and their results showed digestible lysine requirement for broilers based on FCR was higher than that based on BW.

There was a significant effect of the added Met level on feed intake, body weight and feed conversion ratio ($P \leq 0.05$). Adding Met activity up to 0.12% resulted in a significant increase in FI and BW with no further improvement when the added Met level was higher than 0.12%. The FCR was decreased significantly as the supplementary Met activity increased to 0.12%. Adding Met activity with levels from 0.12% to 0.28% had no benefit for FCR. The total digestible Met(dMet) level with 0.16% supplemented Met in basal diet of 0.9% dLys is 0.40%, the total dMet levels with 0.16% supplemented Met in the rest of basal diets is higher than 0.40%. Based on an estimated 88% digestibility of the amino acids in a typical soybean meal diet (Heartland Lysine, 1995), the total Met level, with 0.16% supplemented Met in basal diet of 0.9% dLys, would be approximately 0.45%, which is very close to the NRC (1994) recommendation. Ohta and Ishibashi (1994) suggested the Met requirement of female broilers from 8 to 18 days of age for optimal performance was 0.30% regardless of the dietary TSAA levels.

Results of broken line regression analysis for the estimate of Met requirement are shown in table 6. Estimation was not obtained at every Lys level due to non-convergence. The estimate of dMet level for maximal feed intake at 1.1% Lys level is 0.366±0.021 (mean± SE). At 1.2% Lys level, the estimate of dMet level for optimal feed intake is 0.346±0.131. For optimal BW, the estimate of dMet level at dLys levels of 1.1, 1.2, 1.3, and 1.4% is 0.450±0.023, 0.441± 0.040, 0.536±0.062, and 0.495±0.105, respectively. At dLys levels of 1.1% and 1.2%, the estimated optimal Met levels were higher for BW than those for FI. For optimal FCR, the estimated dMet is 0.439±0.033 at dLys level of 1.0%. The estimates of dMet levels for FI, BW, and FCR at the rest of tested Lys levels could not be converged. These data showed that the optimal dMet levels for FI, BW, and FCR depend on dLys level in the diet.

Broken-line regression analysis for TSAA is shown in table 7. The estimate of digestible TSAA (dTSAA) for maximal FI at dLys level of 0.9, 1.1, and 1.2% is 0.705±0.029, 0.636±0.021, and 0.635±0.098, respectively. For optimal BW, the estimate of dTSAA level at dLys levels of 1.1, 1.2, 1.3, and 1.4% is 0.720±0.023, 0.731±0.040, 0.846±0.026, and 0.825±0.105, respectively. At dLys levels of 1.1% and 1.2%, the estimated optimal dTSAA levels were higher for BW than those for FI. For optimal FCR, the estimate of dTSAA is 0.687±0.076 at Lys level of 0.9%. The estimates of dTSAA levels for FI, BW, and FCR at the rest of tested Lys levels could not be converged (Table 7). Similar to the estimates of the dMet levels, the estimates of dTSAA levels for FI, BW, and FCR depend on dLys level in the diet.

Regression analysis was performed to evaluate the ratio of Met to Lys for optimal performance at each level of Lys increased from 0.9 to 1.4% (Table 8). The results showed that the ratio of Met to Lys for optimal FI at dLys levels of 0.9, 1.1, 1.2, and 1.3% is 52.8±3.2, 42.9±2.9, 28.7±8.1, and 34.7±3.3, respectively. It appears that optimal estimated ratio of Met to Lys decreased as the Lys level increased from 0.9% to 1.2%. For optimal BW, the ratio of Met to Lys at dLys levels of 1.1, 1.2, 1.3, and 1.4% is 41.0±1.9, 36.7±3.4, 41.2±4.6, 35.3±7.5, respectively. The ratios of Met to Lys at different Lys levels changed but with no trend. For optimal FCR, the estimated ratio of Met to Lys at dLys levels of 1.0 and 1.3 is 43.9±3.3 and 30.5 ±4.3, respectively. The estimated ratios of Met to Lys for FI, BW, and FCR at the rest of tested Lys levels could not be converged (Table 8). These variations indicate that the optimal ratios of Met to Lys for FI, BW, and FCR depend on the Lys level in the diet.

The ratios of TSAA to Lys were also estimated with broken-line regression (Table 9). The results showed that the estimated ratio of TSAA to Lys for optimal FI at dLys levels of 0.9, 1.1, 1.2, and 1.3 is 78.3±3.2, 57.8±1.9, 52.9±8.2, 58.5±3.4, respectively. It appears that the optimal estimated ratio of TSAA to Lys decreased as the Lys level increased from 0.9% to 1.2%, which is similar to the ratio of Met to Lys. For optimal BW, the ratio of TSAA to Lys at dLys levels of 1.1, 1.2, 1.3, and 1.4 is 65.4±2.0, 60.9±3.3, 65.0±4.8, 58.9±7.6, respectively. The ratios of TSAA to Lys at different Lys levels changed but with no linear or quadratic trend. For optimal FCR, the estimate of TSAA to Lys ratio at dLys levels of 1.0 and 1.3 is 68.9±3.3 and 54.3±4.3, respectively. The estimated ratios of TSAA to Lys for FI, BW, and FCR at the rest of tested Lys levels could not be converged. These variations of ratios indicate that the optimal ratios of TSAA to Lys for FI, BW, and FCR depend on the Lys level in the diet. However, Knowles and Southern (1998) showed that there were no significant differences ($P > 0.05$) in the estimated ratios of TSAA to Lys required to achieve optimal FI, BW, and FCR for chicks from 4 to 14 d of age fed diets containing two different dietary Lys levels; for chicks fed diets with 1.0% of dLys, the estimated ratios of TSAA to Lys for ADFI, ADG, and FCR were 71, 66, and 63, respectively. The estimated ratios of TSAA to Lys for ADFI, ADG, and FCR for chicks fed diets with 0.82% of dLys were 67, 66, and 63, respectively.

Regardless of Met variation in the diets, regression analysis showed (Table 10) that the optimal dLys requirement for FI, BW, and FCR during this period is 1.215±0.028, 1.132±0.036, and 1.245±0.065, respectively. The requirement of dLys for FCR is higher than these levels for FI and BW. These responses were similar to that of the studies from Zaghari *et al.* (2002) and Garcia *et al.* (2006), who reported that the dLys requirement for optimal FCR was higher than that for BW for broiler chickens in the first several wks.

Significant interactions were observed between Lys and added Met for FI, BW, and FCR ($P \leq 0.05$). This may explain by the fact the optimal Met or TSAA estimates for FI, BW, and FCR depend on the Lys level in the diet and the ratios of Met or TSAA to Lys vary according to the Lys level in the diet. Si *et al.* (2004) reported that when both Met and Lys were fed equal to or in excess of NRC recommendations, there were no significant interactions between Lys and Met for BW, FCR, or breast meat yield. The interactions between Lys and added Met observed in the present study may be because the Met or Lys in some of these diets was not fed sufficiently.

No significant effect of Lys or added Met levels on mortality was observed (data not shown). At the end of this experiment, the total mortality rate was 1.39%.

The results of this study showed that there were significant interactions between Lys and added Met in response to FI, BW, and FCR. The estimated ratios of Met or TSAA to Lys for each of these parameters (FI, BW, and FCR) varied as the dLys levels increased from 0.9 to 1.4%. Therefore, when formulating a diet using Ideal Protein Concept, it is important to know the ideal amino acid profile at a specific dietary Lys levels used in the diet.

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Table 1. Comparison of ideal ratios for amino acids reported in the literature.

*AA	Recommendations for amino acid ratios to lysine by various researchers																ALL AGES		
	**1						2	3	4	5	6	7	8	9	10				
	1-21		22-42		43-56			1-21	20-40	7-28	8-21	0-21	0-42	1-21	10-21	32-43			
	Dig	Total	Dig	Total	Dig	Tot	Dig	Dig	Dig	Dig	Dig	Dig	Dig	TOT	Dig	Dig	MEAN	LO	HI
Lys	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Arg	105	102	105	102	105	102	110	105	112	108		96	105	105	97	101	104	96	112
Gly								65									65		
Gly+Ser		150		140		135								150			144		
His	36	36	36	36	36	36		32		38		24		29	29	31	33	24	38
Ile	65	66	67	68	67	68	70	67	71	63	61.4	65	66	67	64	62	66	61.4	71
Leu	108	108	109	109	109	109		109		108		92		100	94	117	106	92	117
Met	39	39	40	40	40	40				37		38	38	42	35	36	39	35	42
TSAA	71	71	72	72	72	72	75	72	75	70		72	73	75	71	69	72	69	75
Phe	63	63	63	63	63	63				62				60			63		
Phe+Tyr	115	114	115	114	115	114		105		121				112	107	102	112	102	121
Pro								44									44		
Thr	65	68	65	68	65	68	65	67	63	66	55.7	62	65	67	63	65	65	62	68
Trp	16	16	17	17	17	17	18	16	19	14	16.6	18	16	17	16	18	17	14	19
Val	75	76	77	78	77	78	80	77	81	81	77.5	69	80	75	67	75	76	67	81

*AA- amino acid; Dig-digestible; Tot-total; LO-low; HI-high

**1. Rostagno *et al.*, 2005; 2. Schutte and de Jong, 1999; 3. Baker and Han, 1994; 4. Mack *et al.*, 1999; 5. Roth *et al.*, 2001; 6. Baker et al., 2002; 7. Austic, 1994; 8. Dutch Bureau of Livestock Feeding, 1996. 9. NRC 1994 with Lys adjusted to 1.20% and Gly+Ser at 1.80%; 10. Coon, 2004.

Table 2. Composition (g/kg) and calculated nutrient content of diets with different levels of digestible lysine formulated to average ideal ratios of other amino acids except methionine and total sulfur amino acids.

Ingredients	% Digestible Lysine					
	0.90	1.00	1.10	1.20	1.30	1.40
Yellow corn	708.17	648.35	588.52	528.69	468.86	409.02
Poultry oil	4.24	13.70	23.16	32.62	42.07	51.53
Soybean meal	247.53	298.66	349.78	400.90	452.03	503.15
Ground Limestone	3.89	3.51	3.13	2.75	2.37	1.99
Defluorinated phosphate	18.19	17.92	17.66	17.39	17.13	16.87
Sodium chloride	3.65	3.66	3.67	3.68	3.69	3.70
L-Threonine	0.47	0.48	0.50	0.52	0.54	0.56
L-Lysine HCl	2.03	1.89	1.75	1.62	1.48	1.35
Vitamin premix[1]	5.00	5.00	5.00	5.00	5.00	5.00
Trace mineral mix[2]	1.00	1.00	1.00	1.00	1.00	1.00
Pel-Stik[3]	2.50	2.50	2.50	2.50	2.50	2.50
Variable[4]	3.33	3.33	3.33	3.33	3.33	3.33
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
ME kcal/kg	3080.00	3080.00	3080.00	3080.00	3080.00	3080.00
Crude protein %	16.90	18.71	20.53	22.34	24.15	25.97
Calcium %	0.90	0.90	0.90	0.90	0.90	0.90
Nonphytate P, %	0.45	0.45	0.45	0.45	0.45	0.45
Met %	0.32	0.34	0.36	0.38	0.40	0.42
Cys %	0.28	0.31	0.33	0.35	0.38	0.40
Lys %	1.02	1.13	1.24	1.36	1.47	1.59
Thr %	0.68	0.75	0.83	0.91	0.98	1.06
Gly+Ser %	1.52	1.69	1.86	2.03	2.21	2.38
Dig Met %	0.24	0.26	0.27	0.29	0.31	0.33
Dig Cys %	0.23	0.25	0.27	0.29	0.31	0.33
Dig Lys %	0.90	1.00	1.10	1.20	1.30	1.40
Dig Thr %	0.59	0.65	0.71	0.78	0.85	0.91
Dig Arg %	0.98	1.11	1.24	1.37	1.50	1.63
Dig Met + Cys %	0.47	0.51	0.54	0.58	0.62	0.66

[1] Provides per kg of diet: vitamin A (from vitamin A acetate) 7715 IU; cholecalciferol 5511 IU; vitamin E (from dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadione dimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin (from thiamin mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg.
[2] Provides per kg of diet: Mn (from $MnSO_4 \cdot H_20$) 100 mg; Zn (from $ZnSO_4 \cdot 7H_2O$) 100 mg; Fe (from $FeSO_4 \cdot 7H\ _2O$) 50 mg; Cu (from $CuSO_4 \cdot 5H_20$) 10 mg; I from $Ca(IO_3)_2 \cdot H_20$), 1.0 mg.
[3]Uniscope Inc., Johnstown CO 80534.
[4]Variable amounts of MHA-84 and cornstarch.

Table 3. Calculated ratio of digestible amino acids to lysine in basal diets.
(Values in bold italic are at minimum specified level)

Amino Acid	Mean of reports	Digestible Lysine %					
		0.90	1.00	1.10	1.20	1.30	1.40
Lysine	100	100.00	100.00	100.00	100.00	100.00	100.00
Met	39	26.34	25.57	24.94	24.42	23.97	23.59
TSAA	72	52.10	50.65	49.46	48.46	47.63	46.91
Trp	17	18.10	18.67	19.13	19.51	19.84	20.12
Thr	65	***65.00***	***65.00***	***65.00***	***65.00***	***65.00***	***65.00***
Arg	104	108.49	110.70	112.50	114.01	115.28	116.37
Val	76	***76.00***	***76.00***	***76.00***	***76.00***	***76.00***	***76.00***
Ile	66	67.42	68.44	69.26	69.95	70.54	71.04
Leu	106	152.80	147.66	143.45	139.95	136.98	134.44
His	33	43.93	43.68	43.48	43.31	43.16	43.04
Phe	63	83.50	83.30	83.13	82.99	82.87	82.77
Phe+Tyr	112	150.45	150.42	150.40	150.38	150.36	150.34
Gly+Ser	144[1]	150.00	149.62	149.64	149.66	149.68	149.70

[1]Ratio to total lysine.

Table 4 Analyzed crude protein and amino acid contents in basal diets and analyzed MHA activity

Diet ID	dLys0.9	dLys1.0	dLys1.1	dLys1.2	dLys1.3	dLys1.4
Crude Protein	17.7	19.2	21.0	23.0	25.2	26.5
Met %	0.476	0.299	0.319	0.35	0.359	0.382
Cys %	0.478	0.301	0.321	0.348	0.361	0.382
Lys %	0.889	1.188	1.284	1.428	1.51	1.628
Thr %	1.048	0.79	0.865	0.955	0.997	1.081
Gly+Ser %	2.366	1.77	1.916	2.136	2.231	2.414
MHA Activity*	0.326	0.278	0.253	0.238	0.251	0.235

*MHA activity added at the level of 0.3%.

Table 5 Effect of Met and Lys on live performance of 18-d-old broilers

Added Met,%	Dig Lys, %						Mean
	0.9	1.0	1.1	1.2	1.3	1.4	
Feed intake(kg)							
0.00	0.750	0.777	0.785	0.856	0.808	0.780	**0.793^{y}**
0.04	0.726	0.803	0.803	0.855	0.810	0.763	**0.793^{xy}**
0.08	0.760	0.835	0.832	0.846	0.806	0.758	**0.806^{wxy}**
0.12	0.805	0.828	0.840	0.836	0.785	0.816	**0.818^{vw}**
0.16	0.802	0.798	0.838	0.848	0.792	0.788	**0.811^{vwx}**
0.20	0.827	0.825	0.857	0.835	0.826	0.782	**0.825^{v}**
0.24	0.807	0.857	0.835	0.798	0.846	0.775	**0.819^{vw}**
0.28	0.778	0.822	0.828	0.815	0.832	0.795	**0.812^{vwx}**
Mean	**0.782^{c}**	**0.818^{b}**	**0.827^{ab}**	**0.836^{a}**	**0.813^{b}**	**0.782^{c}**	
Body weight(kg)							
0.00	0.502	0.558	0.567	0.657	0.623	0.611	**0.586^{x}**
0.04	0.486	0.582	0.595	0.650	0.642	0.622	**0.596^{x}**
0.08	0.518	0.628	0.628	0.663	0.662	0.622	**0.620^{w}**
0.12	0.595	0.628	0.660	0.666	0.636	0.674	**0.643^{v}**
0.16	0.603	0.610	0.653	0.671	0.632	0.654	**0.637^{v}**
0.20	0.611	0.629	0.659	0.657	0.660	0.650	**0.645^{v}**
0.24	0.587	0.635	0.641	0.643	0.656	0.633	**0.633^{v}**
0.28	0.565	0.616	0.641	0.652	0.673	0.654	**0.634^{v}**
Mean	**0.558^{e}**	**0.611^{d}**	**0.631^{c}**	**0.657^{a}**	**0.648^{ab}**	**0.640^{bc}**	
Feed conversion ratio(kg: kg)							
0.00	1.636	1.508	1.500	1.402	1.399	1.386	**1.472^{v}**
0.04	1.650	1.492	1.458	1.420	1.354	1.318	**1.449^{w}**
0.08	1.608	1.433	1.426	1.378	1.298	1.314	**1.409^{x}**
0.12	1.458	1.422	1.370	1.348	1.324	1.305	**1.371^{z}**
0.16	1.435	1.409	1.370	1.361	1.349	1.293	**1.369^{z}**
0.20	1.463	1.411	1.394	1.374	1.338	1.290	**1.378^{yz}**
0.24	1.491	1.453	1.401	1.335	1.384	1.313	**1.396^{xy}**
0.28	1.492	1.439	1.389	1.350	1.326	1.305	**1.384^{yz}**
Mean	**1.529^{a}**	**1.446^{b}**	**1.413^{c}**	**1.371^{d}**	**1.346^{e}**	**1.315^{f}**	

	Feed intake		Body weight		FCR	
	P-value	SEM	P-value	SEM	P-value	SEM
Lys	<0.0001	0.006	<0.0001	0.004	<0.0001	0.007
Added Met	0.003	0.007	<0.0001	0.005	<0.0001	0.008
Lys x Added Met	0.001	0.021	<0.0001	0.014	<0.0001	0.024

^{abcdef} means in rows with common superscripts do not differ significantly($P \le 0.05$).

^{vwxyz} means in columns with common superscripts do not differ significantly($P \le 0.05$).

Table 6 Estimates of Met requirement at different Lys levels

Variable	parameter	Estimate Met	SE	CI	
				Low	High
FI 18d	Lys0.9	Non convergence			
	Lys1.0	Non convergence			
	Lys1.1	0.366	0.021	0.313	0.419
	Lys1.2	0.346	0.131	-0.019	0.711
	Lys1.3	Non convergence			
	Lys1.4	Non convergence			
BW 18d	Lys0.9	Non convergence			
	Lys1.0	Non convergence			
	Lys1.1	0.450	0.023	0.387	0.512
	Lys1.2	0.441	0.040	0.330	0.551
	Lys1.3	0.536	0.062	0.364	0.707
	Lys1.4	0.495	0.105	0.203	0.788
FCR 18d	Lys0.9	Non convergence			
	Lys1.0	0.439	0.033	0.349	0.529
	Lys1.1	Non convergence			
	Lys1.2	Non convergence			
	Lys1.3	Non convergence			
	Lys1.4	Non convergence			

Table 7 Estimates of TSAA requirement at different Lys levels

Variable	parameter	Estimate TSAA	SE	CI	
				Low	High
FI 18d	Lys0.9	0.705	0.029	0.626	0.785
	Lys1.0	Non convergence			
	Lys1.1	0.636	0.021	0.583	0.689
	Lys1.2	0.635	0.098	0.363	0.906
	Lys1.3	Non convergence			
	Lys1.4	Non convergence			
BW 18d	Lys0.9	Non convergence			
	Lys1.0	Non convergence			
	Lys1.1	0.720	0.023	0.657	0.782
	Lys1.2	0.731	0.040	0.620	0.841
	Lys1.3	0.846	0.062	0.674	1.017
	Lys1.4	0.825	0.105	0.533	1.118
FCR 18d	Lys0.9	0.687	0.076	0.477	0.896
	Lys1.0	Non convergence			
	Lys1.1	Non convergence			
	Lys1.2	Non convergence			
	Lys1.3	Non convergence			
	Lys1.4	Non convergence			

Table 8 Estimates of Met to Lys ratio at different levels of Lys

Variable	parameter	Estimate Met/Lys	SE	CI	
				Low	High
FI 18d	Lys0.9	52.8	3.2	44.0	61.6
	Lys1.0	Non convergence			
	Lys1.1	42.9	2.9	34.9	50.9
	Lys1.2	28.7	8.1	6.1	51.3
	Lys1.3	34.7	3.3	25.5	43.9
	Lys1.4	Non convergence			
BW 18d	Lys0.9	Non convergence			
	Lys1.0	Non convergence			
	Lys1.1	41.0	1.9	35.6	46.4
	Lys1.2	36.7	3.4	27.4	46.0
	Lys1.3	41.2	4.6	28.5	54.0
	Lys1.4	35.3	7.5	14.6	56.0
FCR 18d	Lys0.9	Non convergence			
	Lys1.0	43.9	3.3	34.8	52.9
	Lys1.1	Non convergence			
	Lys1.2	Non convergence			
	Lys1.3	30.5	4.3	18.5	42.4
	Lys1.4	Non convergence			

Table 9 Estimates of TSAA to Lys ratio at different levels of Lys

Variable	parameter	Estimate TSAA/Lys	SE	CI	
				Low	High
FI 18d	Lys0.9	78.3	3.2	69.4	87.2
	Lys1.0	Non convergence			
	Lys1.1	57.8	1.9	52.9	62.6
	Lys1.2	52.9	8.2	30.2	75.6
	Lys1.3	58.5	3.4	49.1	67.9
	Lys1.4	Non convergence			
BW 18d	Lys0.9	Non convergence			
	Lys1.0	Non convergence			
	Lys1.1	65.4	2.0	59.8	71.1
	Lys1.2	60.9	3.3	51.8	70.0
	Lys1.3	65.0	4.8	51.6	78.3
	Lys1.4	58.9	7.6	38.0	79.9
FCR 18d	Lys0.9	Non convergence			
	Lys1.0	68.9	3.3	59.8	77.9
	Lys1.1	Non convergence			
	Lys1.2	Non convergence			
	Lys1.3	54.3	4.3	42.2	66.3
	Lys1.4	Non convergence			

Table 10 Estimates of Lys levels for FI, BW, and FCR

Period	Criteria	Lys Estimate	SE	CI	
				Low	High
0-18 d	FI	1.215	0.028	1.096	1.335
	BW	1.132	0.036	1.020	1.245
	FCR	1.245	0.065	0.965	1.525

Chapter 2 Ratios of Methionine and Total Sulfur Amino Acids to Lysine in Broiler Grower Diets

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ABSTRACT A study was conducted to evaluate the response to Lysine (Lys) and Methionine (Met) in diets on live performance of young broiler chickens during the grower period of 14-35 d. Corn and soybean meal of known protein and moisture content were used to formulate basal diets to provide 0.80 to 1.30% digestible Lys (dLys) in increments of 0.10%. The mean of suggested amino acid ratios to Lys suggested by literature values was used in formulation according to the ideal protein concept. All amino acids other than Met and TSAA were calculated to meet or exceed the expected ratio to Lys. Diets were calculated to be isocaloric with 3142 kcal/kg ME and were supplemented with inorganic trace mineral premix to avoid any source of Met from this premix. Experimental diets were prepared by addition of variable amounts of MHA® (84% of Met) and cornstarch to the Lys basal diets to provide increments of 0.03% up to 0.21% supplemental Met activity for each level of digestible Lys, with a 6 x 8 factorial arrangement of 6 levels of DLys and 8 levels of supplemental Met resulting in a total of 48 treatments. Two consecutive trials using the same experimental diets were conducted with identical design. Two replicate pens were assigned to each dietary treatment in each of the two trials for a total of four replications. Male chicks (Cobb 500) were grown to 14 d on a common diet that was nutritionally complete. At 14 d in each of the two trials, six chicks were assigned to each of 96 compartments in unheated grower battery brooders. Each of the 48 test diets was fed to the two replicate pens of each trial. Body weights by pen were obtained at 14, 28 and 35 d of age with feed consumption determined during the test period. During the period of 14 to 28 d, there were significant effects of dietary Lys levels on feed intake (FI), Body Weight (BW) and feed conversion ratio (FCR)($P \leq 0.05$), with optimal digestible DLys level for FI and BW of 1.08 and 0.91, respectively. The optimal digestible Lys level for FCR is not converged. There were significant effects of added Met levels on BW and FCR ($P \leq 0.05$). During the period of 14 to 35 d, there were significant effects of dietary Lys levels on FI, BW and FCR ($P \leq 0.05$), with optimal dLys level for FI, BW and FCR of 1.20, 1.10 and 1.12, respectively. There were significant effects of added Met levels on BW and FCR ($P \leq 0.05$). No significant interactions between Lys and Met were observed based on FI, BW and FCR during each of

the two periods. There were differences in the estimated ratios of Met or TSAA to Lys required optimizing FI, BW, and FCR for broiler chickens fed different Lys levels. Therefore, the optimal ratios of Met or TSAA to Lys depend on dietary Lys level in the diet. Results of this study suggest that the response to variation in Lys level is independent of Met level, and vice versa in broiler grower diets.

Key words: Broilers, lysine, methionine, ideal protein, live performance

INTRODUCTION

The amino acid requirements of broiler chickens are influenced by a variety of factors, including age, genetic and physiological factors, dietary factors, and environmental factors. It is therefore very difficult to determine the accurate requirement of each individual essential amino acid under various circumstances. In recent years, it has become increasingly popular to formulate diets using the Ideal Protein concept, in which all amino acids are maintained in relation to lysine as the base amino acid. This concept is based on the assumption that the ideal ratio of individual essential amino acids is affected very little by various factors such as genetic, dietary, and environmental factors (Schutte and de Jong, 1999). Formulating diets using this concept will overcome the various internal and external factors when the dLys requirement is determined based on empirical evidence under a specific circumstance and one has access to the database for proper ratios of other essential amino acids to Lys. In addition, this concept minimizes nitrogen pollution to the environment and reduces production cost by preventing over or under fortification of essential amino acids with the use of digestible levels of dietary amino acids (Emmert and Baker, 1997). Numerous reports have suggested various ratios of amino acids to lysine (Table 1). Recommended ratios for methionine range from 35 to 42 per 100 units of lysine with a mean of 39, with TSAA ranging from 69 to 75 with a mean of 72. There is disagreement in the literature regarding the requirement for lysine. When Met and TSAA are held in a ratio to Lys, the concentration of these amino acids increase or decrease as Lys is increased or decreased. Since Met and TSAA are considered the first limiting amino acids in corn-soybean meal diets, the response to variation in Lys may in fact be a response to these amino acids instead. Therefore, this study was conducted to evaluate the separate response to Lys and to Met in diets for young broiler chickens during the grower period of 14-35 d.

MATERIALS AND METHODS

Dietary treatments

Corn and soybean meal of known protein and moisture content were used in formulation of the diets. Amino acid values suggested by Degussa (Degussa AG Feed Additives, 2006), adjusted for the crude protein content of the diet, and amino acid digestion coefficients suggested by Heartland Lysine (Heartland Lysine, 1995) were assigned to the ingredients. The mean of suggested amino acid ratios to Lys suggested by literature values (Table 1) was used in formulation. Diets were formulated to provide 0.80 to 1.30% dLys in increments of 0.10%. All amino acids other than Met and TSAA were calculated to meet or exceed the expected ratio to Lys. Diets were calculated to be isocaloric and were supplemented with complete vitamin and trace mineral premixes obtained from commercial sources. An inorganic trace mineral mix was used so as not to provide any source of methionine from this source.

Experimental diets were prepared by the addition of variable amounts of MHA and cornstarch to the lysine basal diets. MHA (84% dry powder) was added in amounts to provide increments of 0.03% methionine activity up to 0.21% supplemental Met for each level of dLys. The combination of the 6 levels of Lys and 8 levels of added Met resulted in 48 dietary treatments. Following mixing, diets were steam pelleted in a California Pellet Mill Master Model 30 HP pellet mill using a 3/16" (4.76 mm) die.

Birds and management

In two consecutive trials using the same feed mixture, male chicks of a commercial broiler strain (Cobb 500, Cobb-Vantress, Siloam Springs, AR) were obtained from a local hatchery where they had been vaccinated in ovo for Marek's disease and had received vaccinations for Newcastle Disease and Infectious Bronchitis post hatch via a coarse spray. They were grown to 14 d on a common diet that was nutritionally complete. At 14 d in each of the two trials, six chicks were assigned to each of 96 compartments in unheated grower battery brooders with wire floors. Two replicate pens were assigned to each dietary treatment in each of the two studies for a total of four replicates. The experimental diets and tap water were provided for ad libitum consumption. Fluorescent lights provided 24 hr of light daily. Care and management of the birds followed recommended guidelines (FASS, 2010).

Measurements

Body weights by pen were obtained at 14, 28 and 35 d of age with feed consumption determined during the test period. Birds were checked twice daily and any bird that died or was removed to alleviate suffering was weighed with the weight used to adjust feed conversion. A commercial amino acid supplier analyzed samples of the basal diet within each level of dLys for crude protein and total amino acid content. Samples of the diet within each level of dLys with the highest amount of supplemental Met were analyzed for content of supplemental amino acids.

Statistical Analysis

Pen means served as the experimental unit for statistical analysis. Data were subjected to ANOVA as a factorial arrangement of treatments with dietary Lys level and added Met level as the main effects with the interaction of dietary Lys and added Met level using the General Linear Models procedure of SAS (SAS Institute, 1991). When significant differences among treatments were found, means were separated using repeated t-test using the LSMEANS option of the GLM procedure. Mortality data were transformed to $\sqrt{n+1}$ before analysis; Data were presented as natural numbers. Significant statements are based on $p \leq 0.05$.Nonlinear regression analysis was conducted using the PROC LIN procedure of SAS (SAS Institute, 1991) and the SAS macro of Robbins (1986) to determine the level of Met, TSAA, and ratios of Met: Lys and TSAA: Lys that provide the greatest response in FI, BW, and FCR at each level of dietary lysine.

RESULTS AND DISCUSSION

Analyzed crude protein and amino acid contents in the basal diets were in close agreement with the calculated values. The analyzed MHA activity felt in close match to the calculated values (Table 4).

The ANOVA table showing the effects of various levels of dLys and added Met on FI, BW and FCR is shown in table 5 and table 6. During the period of 14 to 28 d, there was a significant effect of dLys level on FI, BW, and

FCR ($P \leq 0.05$). As the dLys levels increased from 0.8 to 1.3%, the FI decreased significantly. The BW increased as the dLys levels increased from 0.8 to 0.9%; there was no improvement of BW to further increase the dLys levels. FCR was reduced in a linear manner as dLys levels increased from 0.8 to 1.3%. As there was no quadratic response of FCR to dLys levels, the dLys requirement for optimal FCR during 14-28 d is no less than 1.3%. During the period of 14 to 35 d, there was a significant effect of dLys level on FI, BW, and FCR ($P \leq 0.05$). As the dLys levels increased from 0.8 to 1.3%, the FI decreased significantly. The BW increased as the dLys levels increased from 0.8 to 1.0 %, there was, however, no improvement of BW to further increase in the dLys levels. FCR was reduced in a linear manner as dLys levels increased from 0.8 to 1.3%. As there was no quadratic response of FCR to dLys levels, the dLys requirement for optimal FCR during 14-35 d is no less than 1.3%.

There was a significant effect of the added Met level on BW and FCR but not on FI ($P \leq 0.05$). During the period of 14 to 28 d, adding Met activity up to 0.09% resulted in a significant increase in BW. FCR decreased significantly as the supplementary Met activity increased to 0.18%. There was no benefit of feed efficiency to further increase in Met level. During the period of 14 to 35 d, adding Met activity up to 0.18% resulted in a significant increase in BW. FCR decreased significantly as the supplementary Met activity increased to 0.18%. The digestible Met (dMet) level in basal diets with 0.8% and 1.3% dLys is 0.22% and 0.31%, respectively. Based on an estimated 88% digestibility of the amino acids in a typical soybean meal diet (Heartland Lysine, 1995), the total Met level in basal diets would be from 0.25 to 0.35%, which is lower than the NRC (1994) recommended level. Therefore, the supplementation of Met to the basal diets improved BW and FCR.

Broken-line regression analysis results (Table 7) showed that, during the period of 14 to 28d, the estimate of dMet level for optimal FI at 0.8% Lys level is 0.286 ± 0.024; at 0.9% Lys level, the estimate of dMet level for optimal FI is 0.340 ± 0.200. The estimate of dMet level for optimal FI at 1.1% Lys level is 0.420 ± 0.047. For optimal BW, the estimate of dMet level at dLys levels of 0.9, 1.0, and 1.1% is 0.378 ± 0.037, 0.437 ± 0.017, and 0.370 ± 0.034, respectively. For optimal FCR, the estimate of dMet level at dLys levels of 0.9, 1.0, 1.1, 1.2, and 1.3 % is 0.375 ± 0.019, 0.395 ± 0.081, 0.367 ± 0.019, 0.396 ± 0.128, and 0.448 ± 0.110, respectively. During the period of 14 to 35d, the estimate of dMet level at dLys levels of 0.8, 0.9, 1.0, and 1.3% for optimal FI is 0.304 ± 0.007, 0.353 ± 0.261, 0.435 ± 0.107, and 0.480 ± 0.033, respectively. For optimal BW, the estimate of dMet level at dLys levels of 0.8, 0.9, 1.0, 1.1, 1.2, and 1.3% is 0.340±0.117, 0.410±0.051, 0.322±0.026, 0.375±0.040, 0.409±0.053,and 0.454±0.044, respectively. For optimal FCR, the estimate of dMet level at dLys levels of 1.1, 1.2, and 1.3 % is 0.424±0.019, 0.482± 0.085, and 0.442±0.048, respectively. The estimates of dMet levels for FI, BW, and FCR at the rest of tested Lys levels during these two periods could not be converged. These data showed that the optimal dMet levels for FI, BW, and FCR depend on dLys level in the diet.

Broken-line regression analysis showed that (Table 8), during the period of 14 to 28 d, the estimate of digestible TSAA (dTSAA) for optimal FI at dLys level of 0.8 and 0.9% is 0.496±0.024, and 0.570±0.200, respectively. For optimal BW, the estimate of dTSAA level at dLys levels of 0.9, 1.0, and 1.1% is 0.595±0.027, 0.683±0.015, and 0.640±0.034, respectively. For optimal FCR, the estimate of dTSAA level at dLys levels of 0.9, 1.1, 1.2, and 1.3% is 0.658±0.058, 0.636±0.018, 0.787±0.035, and 0.756±0.106, respectively. During the period of 14 to 35 d, the estimate of digestible TSAA (dTSAA) for optimal FI at dLys level of 0.8, 0.9, 1.1, and 1.3% is 0.514±0.007, 0.583±0.261, 0.680±0.138, and 0.790±0.033, respectively. For optimal BW, the estimate of dTSAA level at dLys levels of 1.2 and 1.3% is 0.712±0.040 and 0.681±0.030, respectively. For optimal FCR, the estimate of dTSAA level at dLys levels of 0.9 and 1.0% is 0.667±0.032 and 0.656±0.023, respectively. The estimates of dTSAA levels for FI, BW, and FCR at the rest of tested Lys levels during these two periods could not be converged. Similar to the estimates of the dMet levels, the estimates of dTSAA levels for FI, BW, and FCR depend on dLys level in the diet.

Regression analysis was used to evaluate the ratio of Met to Lys for optimal performance at each level of Lys increased from 0.8 to 1.3% (Table 9). During the period of 14 to 28 d, the ratio of Met to Lys for optimal FI at dLys levels of 0.8 and 1.0% is 35.8±3.0 and 32.4±12.5, respectively. For optimal BW, the ratio of Met to Lys at dLys levels of 0.9 and 1.1% is 40.6±3.0, and 33.7±3.1, respectively. It appears that optimal ratio of Met to Lys decreased as the Lys level increased. For optimal FCR, the estimated ratio of Met to Lys at dLys levels of 0.9 and 1.0 is 31.5±4.0, and 40.4±7.9, respectively. Contrast to the response of FI and BW, for optimal FCR, the ratio of Met to Lys increased as the Lys level increased. During the period of 14 to 35 d, the ratio of Met to Lys for optimal FI at dLys levels of 0.8, 0.9, 1.1, and 1.3% is 33.3±0.905, 39.3±30.122, 37.3±12.827, and 29.2±11.650, respectively. For optimal BW, the estimated ratio of Met to Lys at dLys levels of 0.8, 0.9, 1.1, 1.2, and 1.3% is 49.3±6.816, 45.0±7.733, 31.3±2.288, 35.2±3.365, and 36.7±1.261, respectively. For optimal FCR, the ratio of Met to Lys at dLys levels of 0.9, 1.1, and 1.3% is 48.6±3.599, 36.4±1.304, and 31.2±14.498, respectively. It appears that the ratio of Met to Lys for optimal FCR decreased as the Lys level increased. Research conducted by Coon (2004) showed that the ideal Met: Lys ratio based on broken line analysis for BW and FCR with 1.2% dLys was 35 and 36, respectively, which is similar to the present study. These variations indicate that the optimal ratios of Met to Lys for FI, BW, and FCR depend on the Lys level in the diet. The estimated ratios of Met to Lys for FI, BW, and FCR at the rest of tested dLys levels could not be converged

The ratios of TSAA to Lys were also estimated with broken-line regression (Table 10). The results showed that, during the period of 14 to 28 d, the estimated ratio of TSAA to Lys for optimal FI at dLys levels of 0.8, 1.0, and 1.3% is 61.9±3.151, 57.4±12.454, 55.6±35.206, respectively. It appears that the estimated ratio of TSAA to Lys for optimal FI decreased as the Lys level increased, which is similar to the ratio of Met to Lys for optimal FI during the period of 14 to 28 d. For optimal BW, the ratio of TSAA to Lys at dLys levels of 1.0, 1.1, and 1.3% is 68.5±1.297, 58.2±3.033, and 50.6±6.381, respectively. The ratios of TSAA to Lys for optimal BW decreased as the dLys level increased. For optimal FCR, the estimate of TSAA to Lys ratio at dLys levels of 0.9, 1.0, 1.1, and 1.2% is 72.9±6.274, 65.4±7.883, 57.4±3.302, 56.1±4.013, respectively. During the period of 14 to 35 d, the estimated ratio of TSAA to Lys for optimal FI at dLys levels of 0.8, 0.9, and 1.0% is 64.2±1.099, 64.8±30.122, and 64.0±19.974, respectively. It appears that the estimated ratio of TSAA to Lys for optimal FI does not vary as the Lys level increases. For optimal BW, the ratio of TSAA to Lys at dLys levels of 1.0, 1.1, and 1.3% is 55.8±2.288, 59.3±3.365, and 60.4±1.275, respectively. The ratios of TSAA to Lys for optimal BW increased as the dLys level increased. These ratios were lower than the one reported by Mack et al. (1999). They estimated that when the digestible Lys required for BW was 0.86% in the diet, the ideal ratio of TSAA to Lys was 75 based on BW gain. However, Vieira et al. (2004) demonstrated that optimum TSAA: Lys ratio for growing broilers based on breast meat yield and FCR might be higher than 77. In addition, they suggested that the optimum dietary TSAA level depends on dietary protein level and thus should be related to the protein level in the diet. For optimal FCR, the estimate of TSAA to Lys ratio at dLys levels of 1.0, 1.1, and 1.3% is 65.6±2.282, 67.9±3.579, and 55.7±8.059, respectively. The estimated ratios of TSAA to Lys for FI, BW, and FCR at the rest of tested dLys levels could not be converged. Research conducted by Coon (2004) showed that the ideal TSAA: Lys ratio based on broken line analysis for BW and FCR with 1.2% dLys was 67 and 70, respectively. According to Schutte and de Jong (1999), a higher ideal TSAA: Lys ratio was estimated, which was approximately 75. These variations of ratios indicate that the optimal ratios of TSAA to Lys for FI, BW, and FCR depend on the Lys level in the diet.

Regardless of Met variation in the diets, regression analysis shows that during the period of 14 to 28 d, the optimal dLys requirement for FI and BW is 1.08±0.122, and 0.91±0.013, respectively. The requirement for FCR could not be converged. During the period of 14 to 35 d, the optimal dLys requirement for FI, BW, and FCR is 1.20±0.068, 1.10±0.095, and 1.12±0.055, respectively. The estimate of dLys requirement for FCR was very close to the estimation by Mack et al. (1999), who reported that the true fecal digestible Lys requirement for FCR was 1.15% for broiler chickens of 20 to 40 d of age. During both periods, the dLys requirement for FI is higher than the requirement for BW and FCR. The requirement of dLys for BW and FCR is similar; however studies from Baker *et al.* (2002) and Garcia et al. (2006) showed that the dLys requirement for optimal FCR was higher than that for maximal BW gain for broiler chickens.

No significant interactions were observed between Lys and added Met for FI, BW, and FCR ($P>0.05$). Similarly, Si *et al.* (2004) reported that when both Met and Lys were fed equal to or in excess of NRC recommendations, there were no significant interactions between Lys and Met for BW, FCR, or breast meat yield.

No significant effect of Lys or added Met levels on mortality was observed (data not shown). During the experimental period of 14 to 35 d, the total mortality rate was 2.34%.

The results of this study showed that the optimal ratios of Met or TSAA to Lys for each of these parameters (FI, BW, and FCR) vary as the dLys levels increased from 0.8 to 1.3%. Therefore, when formulating a diet using Ideal Protein Concept, it is important to know the ideal amino acid profile at a specific dietary Lys levels used in the diet.

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Table 1. Comparison of Ideal ratios for amino acids reported in the literature.

*AA	Recommendations for amino acid ratios to lysine by various researchers																ALL AGES		
	**1						2	3	4	5	6	7	8	9	10				
	1-21		22-42		43-56			1-21	20-40	7-28	8-21	0-21	0-42	1-21	10-21	32-43			
	Dig	Total	Dig	Total	Dig	Tot	Dig	Dig	Dig	Dig	Dig	Dig	Dig	Tot	Dig	Dig	MEAN	LO	HI
Lys	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Arg	105	102	105	102	105	102	110	105	112	108		96	105	105	97	101	104	96	112
Gly								65									65		
Gly+Ser		150		140		135								150			144		
His	36	36	36	36	36	36		32		38		24		29	29	31	33	24	38
Ile	65	66	67	68	67	68	70	67	71	63	61.4	65	66	67	64	62	66	61.4	71
Leu	108	108	109	109	109	109		109		108		92		100	94	117	106	92	117
Met	39	39	40	40	40	40				37		38	38	42	35	36	39	35	42
TSAA	71	71	72	72	72	72	75	72	75	70		72	73	75	71	69	72	69	75
Phe	63	63	63	63	63	63				62				60			63		
Phe+Tyr	115	114	115	114	115	114		105		121				112	107	102	112	102	121
Pro								44									44		
Thr	65	68	65	68	65	68	65	67	63	66	55.7	62	65	67	63	65	65	62	68
Trp	16	16	17	17	17	17	18	16	19	14	16.6	18	16	17	16	18	17	14	19
Val	75	76	77	78	77	78	80	77	81	81	77.5	69	80	75	67	75	76	67	81

*AA- amino acid; Dig-digestible; Tot-total; LO-low; HI-high

**1. Rostagno *et al.*, 2005; 2. Schutte and de Jong, 1999; 3. Baker and Han, 1994; 4. Mack *et al.*, 1999; 5. Roth *et al.*, 2001; 6. Baker et al., 2002; 7. Austic, 1994; 8. Dutch Bureau of Livestock Feeding, 1996. 9. NRC 1994 with Lys adjusted to 1.20% and Gly+Ser at 1.80%; 10. Coon, 2004.

Table 2. Composition (g/kg) and calculated nutrient content of diets with different levels of dLys formulated to average ideal ratios of other amino acids except methionine and total sulfur amino acids.

Ingredients	% DLys					
	0.80	0.90	1.00	1.10	1.20	1.30
Yellow corn	762.92	703.08	643.25	583.44	552.02	498.48
Poultry oil	2.78	12.23	21.69	31.15	39.77	47.18
Soybean meal	197.08	248.20	299.33	350.45	373.63	420.44
Ground Limestone	6.45	6.07	5.69	5.30	5.07	4.73
Defluorinated phosphate	12.90	12.65	12.38	12.12	12.02	11.76
Sodium chloride	4.28	4.29	4.30	4.30	4.31	4.32
L-Threonine	0.44	0.46	0.48	0.50	0.52	0.54
L-Lysine HCl	2.15	2.02	1.88	1.74	1.66	1.55
Vitamin premix[1]	5.00	5.00	5.00	5.00	5.00	5.00
Trace mineral mix[2]	1.00	1.00	1.00	1.00	1.00	1.00
Pel-Stik[3]	2.50	2.50	2.50	2.50	2.50	2.50
Variable[4]	2.50	2.50	2.50	2.50	2.50	2.50
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
ME kcal/kg	3135.00	3135.00	3135.00	3135.00	3135.00	3135.00
Crude protein %	15.08	16.90	18.71	20.52	22.10	23.85
Calcium %	0.80	0.80	0.80	0.80	0.80	0.80
Nonphytate P, %	0.35	0.35	0.35	0.35	0.35	0.35
Met %	0.24	0.26	0.28	0.30	0.38	0.40
Cys %	0.26	0.28	0.31	0.33	0.35	0.38
Lys %	0.90	1.02	1.13	1.24	1.35	1.46
Thr %	0.60	0.68	0.75	0.83	0.90	0.97
Gly+Ser %	1.35	1.52	1.69	1.86	2.02	2.19
Dig Met %	0.22	0.24	0.26	0.27	0.29	0.31
Dig Cys %	0.21	0.23	0.25	0.27	0.29	0.31
Dig Lys %	0.80	0.90	1.00	1.10	1.20	1.30
Dig Thr %	0.52	0.59	0.65	0.72	0.78	0.85
Dig Arg %	0.85	0.98	1.11	1.24	1.37	1.49
Dig Met + Cys %	0.43	0.47	0.51	0.54	0.58	0.62

[1] Provides per kg of diet: vitamin A (from vitamin A acetate) 7715 IU; cholecalciferol 5511 IU; vitamin E (from dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadionedimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin (from thiamin mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg.
[2] Provides per kg of diet: Mn (from $MnSO_4 \cdot H_20$) 100 mg; Zn (from $ZnSO_4 \cdot 7H_2O$) 100 mg; Fe (from $FeSO_4 \cdot 7H\ _2O$) 50 mg; Cu (from $CuSO_4 \cdot 5H_20$) 10 mg; I from $Ca(IO_3)_2 \cdot H_20$), 1.0 mg.
[3]Uniscope Inc., Johnstown CO 80534.
[4]Variable amounts of MHA-84 and cornstarch.

Table 3 Calculated ratios of digestible amino acids to lysine in basal diets.

(Values in bold italic are at minimum specified levels).

Amino Acid	Mean of reports	Digestible Lysine %					
		0.80	0.90	1.00	1.10	1.20	1.30
Lysine	100	100.00	100.00	100.00	100.00	100.00	100.00
Met	39	27.3	26.3	25.6	24.9	24.5	24.1
TSAA	72	53.9	52.1	50.6	49.4	48.6	47.8
Trp	17	17.4	18.12	18.7	19.1	19.5	19.8
Thr	65	***65.0***	***65.0***	***65.0***	***65.0***	***65.0***	***65.00***
Arg	104	105.8	108.5	110.76	112.5	113.8	114.9
Val	76	***76.0***	***76.0***	***76.0***	***76.0***	***76.0***	***76.00***
Ile	66	66.2	67.5	68.5	69.3	69.8	70.4
Leu	106	159.0	152.6.	147.5	143.3	140.5	137.7
His	33	44.2	43.9	43.7	43.5	43.3	43.2
Phe	63	83.8	83.5	83.3	83.1	83.0	82.9
Phe+Tyr	112	150.5	150.4	150.4	150.4	147.6	147.44
Gly+Ser	144[1]	149.6	149.5	147.5	149.6	149.8	149.84

[1]Ratio to total lysine

Table 4 Analyzed crude protein and amino acid contents in basal diets

Diet ID	dLys0.8	dLys0.9	dLys1.0	dLys1.1	dLys1.2	dLys1.3
Crude Protein	15.4	18.3	20.2	20.8	23.6	24.9
Met %	0.293	0.301	0.313	0.339	0.359	0.378
Cys %	0.243	0.275	0.291	0.310	0.330	0.345
Lys %	0.889	1.118	1.183	1.288	1.383	1.474
Thr %	0.613	0.725	0.779	0.862	0.916	0.966
Gly+Ser %	1.354	1.571	1.699	1.865	1.981	2.121

Table 5 Effect of Lys and added Met on live performance of 14-28d-old broilers

Added Met,%	Dig Lys, %						Mean
	0.8	0.9	1.0	1.1	1.2	1.3	
Feed intake							
0.00	1.796	1.667	1.710	1.638	1.544	1.555	1.651
0.03	1.723	1.738	1.658	1.698	1.658	1.594	1.678
0.06	1.689	1.741	1.613	1.651	1.535	1.571	1.633
0.09	1.695	1.667	1.704	1.639	1.653	1.555	1.652
0.12	1.694	1.693	1.539	1.566	1.609	1.547	1.608
0.15	1.755	1.686	1.661	1.566	1.574	1.589	1.639
0.18	1.739	1.706	1.617	1.601	1.629	1.599	1.648
0.21	1.728	1.659	1.643	1.631	1.609	1.522	1.632
Mean	1.727^{x}	1.695^{xy}	1.643^{yz}	1.624^{zw}	1.601^{zw}	1.567^{w}	
Body weight(kg)							
0.00	1.340	1.313	1.370	1.337	1.327	1.374	1.343^{e}
0.03	1.252	1.386	1.401	1.382	1.326	1.390	1.356^{de}
0.06	1.327	1.383	1.395	1.396	1.365	1.407	1.379^{dc}
0.09	1.389	1.390	1.389	1.423	1.391	1.393	1.396^{abc}
0.12	1.380	1.410	1.392	1.414	1.404	1.408	1.401^{abc}
0.15	1.302	1.423	1.378	1.399	1.435	1.411	1.391^{bc}
0.18	1.376	1.414	1.397	1.427	1.459	1.451	1.421^{ab}
0.21	1.387	1.421	1.437	1.405	1.462	1.415	1.421^{a}
Mean	1.344^{y}	1.393^{x}	1.395^{x}	1.398^{x}	1.396^{x}	1.406^{x}	
Feed conversion ratio(kg: kg)							
0.00	1.938	1.852	1.783	1.794	1.800	1.609	1.796^{a}
0.03	1.978	1.798	1.683	1.737	1.812	1.636	1.774^{a}
0.06	1.925	1.783	1.646	1.644	1.668	1.553	1.703^{b}
0.09	1.806	1.764	1.690	1.655	1.693	1.628	1.706^{b}
0.12	1.845	1.713	1.682	1.634	1.617	1.555	1.674^{bc}
0.15	1.862	1.686	1.687	1.641	1.625	1.591	1.682^{b}
0.18	1.813	1.720	1.633	1.584	1.557	1.530	1.639^{c}
0.21	1.784	1.717	1.607	1.623	1.521	1.579	1.639^{c}
Mean	1.869^{x}	1.754^{y}	1.677^{z}	1.664^{z}	1.661^{z}	1.585^{w}	

	Feed intake		Body weight		FCR	
	P-value	SEM	P-value	SEM	P-value	SEM
Lys	<0.0001	0.024	<0.0001	0.009	<0.0001	0.014
Added Met	0.766	0.028	<0.0001	0.011	<0.0001	0.017
Lys x Added Met	1.000	0.074	0.427	0.035	0.233	0.063

[abcde] means in columns with common superscripts do not differ significantly(p≤0.05).

[xyzw] means in rows with common superscripts do not differ significantly(p≤0.05).

Table 6 Effect of Lys and added Met on live performance of 14-35d-old broilers

Added Met,%	Dig Lys, %						Mean
	0.8	0.9	1.0	1.1	1.2	1.3	
Feed intake							
0.00	3.125	2.893	2.973	2.787	2.732	2.728	2.873
0.03	2.940	3.034	2.880	2.966	2.906	2.820	2.924
0.06	2.891	2.964	2.746	2.873	2.667	2.731	2.812
0.09	2.849	2.893	3.031	2.848	2.938	2.733	2.882
0.12	2.892	2.928	2.710	2.763	2.790	2.699	2.797
0.15	2.951	2.895	2.865	2.779	2.759	2.916	2.861
0.18	2.977	2.914	2.817	2.815	2.860	2.787	2.862
0.21	3.009	2.865	2.861	2.816	2.804	2.677	2.839
Mean	2.954^{a}	2.923^{ab}	2.860^{bc}	2.831^{bcd}	2.807^{cd}	2.761^{d}	
Body weight(kg)							
0.00	1.995	1.968	2.029	2.031	1.998	2.024	2.007^{w}
0.03	1.887	2.061	2.047	2.132	1.980	2.073	2.030^{zw}
0.06	1.968	2.070	2.125	2.103	2.049	2.086	2.067^{yz}
0.09	2.036	2.023	2.134	2.147	2.088	2.088	2.086^{y}
0.12	2.046	2.068	2.106	2.139	2.070	2.121	2.092^{y}
0.15	1.987	2.095	2.088	2.147	2.085	2.153	2.093^{y}
0.18	2.026	2.069	2.095	2.139	2.197	2.161	2.115^{xy}
0.21	2.073	2.130	2.184	2.139	2.174	2.158	2.143^{x}
Mean	2.002^{c}	2.060^{b}	2.101^{a}	2.122^{a}	2.08^{ab}	2.108^{a}	
Feed conversion ratio(kg: kg)							
0.00	1.968	1.895	1.840	1.827	1.793	1.687	1.835^{x}
0.03	1.986	1.852	1.772	1.761	1.850	1.702	1.820^{x}
0.06	1.947	1.838	1.754	1.724	1.731	1.640	1.772^{y}
0.09	1.874	1.838	1.747	1.693	1.733	1.650	1.756^{y}
0.12	1.864	1.795	1.706	1.677	1.678	1.621	1.724^{z}
0.15	1.909	1.749	1.708	1.666	1.653	1.647	1.722^{z}
0.18	1.854	1.774	1.672	1.632	1.603	1.588	1.687^{w}
0.21	1.822	1.742	1.645	1.655	1.598	1.603	1.677^{w}
Mean	1.903^{a}	1.810^{b}	1.731^{c}	1.704^{d}	1.705^{dc}	1.642^{e}	

	Feed intake		Body weight		FCR	
	P-value	SEM	P-value	SEM	P-value	SEM
Lys	0.0004	0.034	<0.0001	0.016	<0.0001	0.009
Added Met	0.339	0.040	<0.0001	0.020	<0.0001	0.012
Lys x Added Met	0.903	0.106	0.868	0.077	0.654	0.049

[abcde] means in rows with common superscripts do not differ significantly(p≤0.05).

[xyzw] means in columns with common superscripts do not differ significantly($p \le 0.05$).

Table 7 Estimates of digestible Met requirement at different Lys levels

Variable	parameter	Met Estimate	SE	CI	
				Low	High
FI 14-28d	Lys0.8	0.286	0.024	0.220	0.353
	Lys0.9	0.340	0.200	0.000	0.896
	Lys1.0	Non convergence			
	Lys1.1	0.420	0.047	0.289	0.552
	Lys1.2	Non convergence			
	Lys1.3	Non convergence			
BW 14-28d	Lys0.8	Non convergence			
	Lys0.9	0.378	0.037	0.283	0.473
	Lys1.0	0.437	0.017	0.390	0.484
	Lys1.1	0.370	0.034	0.277	0.463
	Lys1.2	Non convergence			
	Lys1.3	Non convergence			
FCR 14-28d	Lys0.8	Non convergence			
	Lys0.9	0.375	0.019	0.326	0.424
	Lys1.0	0.395	0.081	0.188	0.602
	Lys1.1	0.367	0.019	0.319	0.415
	Lys1.2	0.396	0.128	0.040	0.752
	Lys1.3	0.448	0.110	0.165	0.732
FI 14-35d	Lys0.8	0.304	0.007	0.285	0.324
	Lys0.9	0.353	0.261	0.000	1.077
	Lys1.0	0.435	0.107	0.138	0.733
	Lys1.1	Non convergence			
	Lys1.2	Non convergence			
	Lys1.3	0.480	0.033	0.390	0.570
BW 14-35d	Lys0.8	0.340	0.117	0.039	0.641
	Lys0.9	0.410	0.051	0.268	0.552
	Lys1.0	0.322	0.026	0.255	0.389
	Lys1.1	0.375	0.040	0.273	0.477
	Lys1.2	0.409	0.053	0.274	0.544
	Lys1.3	0.454	0.044	0.342	0.567
FCR 14-35d	Lys0.8	Non convergence			
	Lys0.9	Non convergence			
	Lys1.0	Non convergence			
	Lys1.1	0.424	0.019	0.375	0.474
	Lys1.2	0.482	0.085	0.264	0.700
	Lys1.3	0.442	0.048	0.319	0.565

Table 8 Estimates of digestible TSAA requirement at different Lys levels

Variable	parameter	TSAA Estimate	SE	CI	
				Low	High
FI 14-28d	Lys0.8	0.496	0.024	0.430	0.563
	Lys0.9	0.570	0.200	0.014	1.126
	Lys1.0	Non convergence			
	Lys1.1	Non convergence			
	Lys1.2	Non convergence			
	Lys1.3	Non convergence			
BW 14-28d	Lys0.8	Non convergence			
	Lys0.9	0.595	0.027	0.525	0.665
	Lys1.0	0.683	0.015	0.642	0.724
	Lys1.1	0.640	0.034	0.547	0.733
	Lys1.2	Non convergence			
	Lys1.3	Non convergence			
FCR 14-28d	Lys0.8	Non convergence			
	Lys0.9	0.658	0.058	0.498	0.819
	Lys1.0	Non convergence			
	Lys1.1	0.636	0.018	0.591	0.681
	Lys1.2	0.787	0.035	0.698	0.876
	Lys1.3	0.756	0.106	0.485	1.028
FI 14-35d	Lys0.8	0.514	0.007	0.495	0.534
	Lys0.9	0.583	0.261	0.000	1.307
	Lys1.0	Non convergence			
	Lys1.1	0.680	0.138	0.324	1.035
	Lys1.2	Non convergence			
	Lys1.3	0.790	0.033	0.700	0.880
BW 14-35d	Lys0.8	Non convergence			
	Lys0.9	Non convergence			
	Lys1.0	Non convergence			
	Lys1.1	Non convergence			
	Lys1.2	0.712	0.040	0.600	0.824
	Lys1.3	0.681	0.030	0.599	0.763
FCR 14-35d	Lys0.8	Non convergence			
	Lys0.9	0.667	0.032	0.585	0.750
	Lys1.0	0.656	0.023	0.592	0.719
	Lys1.1	Non convergence			
	Lys1.2	Non convergence			
	Lys1.3	Non convergence			

Table 9 Estimates of Met/Lys ratio at different Lys levels

Variable	parameter	Met/Lys ratio	SE	CI	
				Low	High
FI 14-28d	Lys0.8	35.8	3.0	27.5	44.2
	Lys0.9	Non convergence			
	Lys1.0	32.4	12.5	0.0	67.0
	Lys1.1	Non convergence			
	Lys1.2	Non convergence			
	Lys1.3	Non convergence			
BW 14-28d	Lys0.8	Non convergence			
	Lys0.9	40.6	3.0	32.8	48.4
	Lys1.0	Non convergence			
	Lys1.1	33.7	3.1	25.2	42.2
	Lys1.2	Non convergence			
	Lys1.3	Non convergence			
FCR 14-28d	Lys0.8	Non convergence			
	Lys0.9	31.5	4.0	20.5	42.6
	Lys1.0	40.4	7.9	20.1	60.6
	Lys1.1	Non convergence			
	Lys1.2	Non convergence			
	Lys1.3	Non convergence			
FI 14-35d	Lys0.8	33.3	0.905	30.818	35.841
	Lys0.9	39.3	30.122	0.000	122.900
	Lys1.0	Non convergence			
	Lys1.1	37.3	12.827	4.371	70.314
	Lys1.2	Non convergence			
	Lys1.3	29.2	11.650	0.000	328.700
BW 14-35d	Lys0.8	49.3	6.816	30.359	68.205
	Lys0.9	45.0	7.733	25.097	64.850
	Lys1.0	Non convergence			
	Lys1.1	31.3	2.288	25.432	37.193
	Lys1.2	35.2	3.365	25.879	44.566
	Lys1.3	36.7	1.261	33.415	39.900
FCR 14-35d	Lys0.8	Non convergence			
	Lys0.9	48.6	3.599	39.348	57.851
	Lys1.0	Non convergence			
	Lys1.1	36.4	1.304	33.005	39.709
	Lys1.2	Non convergence			
	Lys1.3	31.2	14.498	0.000	71.482

Table 10 Estimates of TSAA/Lys ratio at different Lys levels

Variable	parameter	TSAA/Lys ratio	SE	CI	
				Low	High
FI 14-28d	Lys0.8	61.9	3.151	53.168	70.667
	Lys0.9	Non convergence			
	Lys1.0	57.4	12.454	22.868	92.021
	Lys1.1	Non convergence			
	Lys1.2	Non convergence			
	Lys1.3	55.6	35.206	0.000	146.100
BW 14-28d	Lys0.8	Non convergence			
	Lys0.9	Non convergence			
	Lys1.0	68.5	1.297	64.856	72.060
	Lys1.1	58.2	3.033	49.806	66.646
	Lys1.2	Non convergence			
	Lys1.3	50.6	6.381	32.863	68.295
FCR 14-28d	Lys0.8	Non convergence			
	Lys0.9	72.9	6.274	55.521	90.361
	Lys1.0	65.4	7.883	45.115	85.644
	Lys1.1	57.4	3.302	48.246	66.579
	Lys1.2	56.1	4.013	44.947	67.231
	Lys1.3	Non convergence			
FI 14-35d	Lys0.8	64.2	1.099	61.159	67.259
	Lys0.9	64.8	30.122	0.000	148.400
	Lys1.0	64.0	19.974	8.510	119.400
	Lys1.1	Non convergence			
	Lys1.2	Non convergence			
	Lys1.3	Non convergence			
BW 14-35d	Lys0.8	Non convergence			
	Lys0.9	Non convergence			
	Lys1.0	Non convergence			
	Lys1.1	55.8	2.288	49.932	61.693
	Lys1.2	59.3	3.365	49.979	68.666
	Lys1.3	60.4	1.275	57.161	63.715
FCR 14-35d	Lys0.8	Non convergence			
	Lys0.9	Non convergence			
	Lys1.0	65.6	2.282	59.232	71.905
	Lys1.1	67.9	3.579	57.996	77.870
	Lys1.2	Non convergence			
	Lys1.3	55.7	8.059	33.318	78.065

Table 11 Estimates of Lys levels at different growing periods

Period	Criteria	Lys Estimate	SE	CI	
				Low	High
14-28 d	FI	1.08	0.122	0.554	1.602
	BW	0.91	0.013	0.872	0.952
	FCR	Non convergence			
14-35 d	FI	1.20	0.068	0.985	1.415
	BW	1.10	0.095	0.689	1.508
	FCR	1.12	0.055	0.943	1.293

Chapter 3 Ratios of Methionine and Total Sulfur Amino Acids to Lysine in Broiler Finisher Diets

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ABSTRACT A study was conducted to evaluate the response to Lysine (Lys) and Methionine (Met) in diets on performance of broiler chickens during the finisher period of 35-49 d. The ratios of Met: Lys and TSAA: Lys that provide the greatest live performance response were also determined. Corn and soybean meal of known protein and moisture content were used to formulate basal diets to provide 0.60 to 1.1% digestible Lys (dLys) in increments of 0.10%. The mean of suggested amino acid ratios to Lys suggested by literature values was used in formulation according to the ideal protein concept. All amino acids other than Met and TSAA were calculated to meet or exceed the expected ratio to Lys. Diets were calculated to be isocaloric with 3100 kcal/kg ME and were supplemented with inorganic trace mineral premix to avoid any source of Met from this premix. Experimental diets were prepared by addition of variable amounts of DL methionine or inert filler to each of the six lysine basal diets to provide 0.00, 0.10, 0.20, and 0.40% additional methionine for each level of Lys, resulting in a total of 24 treatments. Male chicks (Cobb 500) were grown to 35 d on a nutritionally complete diet. At 35 d, five chicks were assigned to each of 144 compartments in finisher battery brooders. Each of the 24 test diets was fed to six replicate pens. Body weights by pen were obtained at 35 and 49 d of age with feed consumption determined during the test period. There were significant effects of dietary Lys levels on body weight gain (BWG) and feed conversion ratio (FCR), with optimal dLys level for BWG and FCR of 1.01 and 1.05, respectively. There was a significant effect of supplemental Met on FCR. No significant interactions were observed between Lys and supplemental Met on feed intake (FI), BWG, and FCR. Increasing Lys level significantly improved dressing percentage and breast meat yield. There were differences in the estimated ratios of Met or TSAA to Lys required for optimizing FI, BWG, and FCR for broiler chickens fed different Lys levels. Therefore, the optimal ratios of Met or TSAA to Lys depend on dietary Lys level in the diet. Results of this study suggest that the response to variation in Lys level is independent of Met level, and vice versa in broiler finisher diets. The ideal amino acid profile may depend on the Lys level in the diets.

Key words: Broilers, lysine, methionine, ideal protein, live performance

INTRODUCTION

The amino acid requirements of broiler chickens are influenced by a variety of factors, including age, genetic and physiological factors, dietary factors, and environmental factors. It is therefore very difficult to determine the accurate requirement of each individual essential amino acid under various circumstances. In recent years, it has become increasingly popular to formulate diets using the Ideal Protein concept in which all amino acids are maintained in relation to lysine as the base amino acid. This concept is based on the assumption that the ideal ratio of individual essential amino acid is affected very little by various factors such as genetic, dietary, and environmental factors (Schutte and de Jong, 1999). Formulating diets using this concept will overcome the various internal and external factors when the dLys requirement is determined based on empirical evidence under a specific circumstance and one has access to the database for proper ratios of other essential amino acids to Lys. In addition, this concept minimizes nitrogen pollution to environment and reduces production cost by preventing over or under fortification of essential amino acids with the use of digestible levels of dietary amino acids (Emmert and Baker, 1997). Numerous reports have suggested various ratios of amino acids to lysine (Table 1). Recommended ratios for methionine range from 35 to 42 per 100 units of lysine with a mean of 39, with TSAA ranging from 69 to 75 with a mean of 72. There is disagreement in the literature regarding the requirement for lysine. When Met and TSAA are held in a ratio to Lys, the concentration of these amino acids increase or decrease as Lys is increased or decreased. The previous studies in our lab showed that the ideal ratios of Met or TSAA to Lys depend on the Lys level in the diet. Even in diets with the same level of lysine, the optimum ratios of Met or TSAA to Lys are different at different growing stages. Since Met and TSAA are considered the primary limiting amino acids in corn-soybean meal diets, the response to variation in Lys may in fact be a response to these amino acids instead. Therefore, this study was conducted to evaluate the response of increased ratios of Met or TSAA to Lys at each level of lysine in diets for broiler chickens during the finisher period of 35 to 49 d of age.

MATERIALS AND METHODS

Dietary treatments

Corn and soybean meal of known protein and moisture content were used in formulation of the diets. Amino acid values suggested by Degussa (Degussa AG Feed Additives, 2006), adjusted for the crude protein content of the diet, and amino acid digestion coefficients suggested by Heartland Lysine (Heartland Lysine, 1995) were assigned to the ingredients. The mean of suggested amino acid ratios to Lys suggested by literature values (Table 1) was used in formulation. Diets were formulated to provide 0.60 to 1.10% dLys in increments of 0.10%. All amino acids, other than Met and TSAA, were calculated to meet or exceed the expected ratio to Lys. Diets were calculated to be isocaloric with 3100 ME kcal/kg and were supplemented with complete vitamin and trace mineral premixes obtained from commercial sources. An inorganic trace mineral mix was used so as not to provide any source of methionine from this source. Allowances were made in the formulas for addition of DL methionine or inert filler. The composition of the resulting diets is shown in Table 2 with the calculated nutrient content shown in Table 3.

Experimental diets were prepared by addition of variable amounts of DL methionine or inert filler to each of the six lysine basal diets to provide 0.00, 0.10, 0.20, and 0.40% additional methionine for each level of Lys.These levels will provide sufficient methionine to meet or exceed the levels suggested in the composite Ideal Ratio (Table 4). This resulted in a total of 24 dietary treatments.

Birds and management

Male chicks of a commercial broiler strain (Cobb 500, Cobb-Vantress, Siloam Springs, AR) were obtained from a local hatchery where they had been vaccinated in ovo for Marek's disease and had received vaccinations for Newcastle Disease and Infectious Bronchitis post hatch via a coarse spray. These male broilers were grown to 35 d on nutritionally complete starter and grower diets. A total of 720 birds were randomly assigned to 144-compartments in wire floored finishing batteries maintained in a temperature-controlled environment. Each dietary treatment was fed to 6 replicate pens of 5 male broilers from 35 to 49 d of age. The experimental diets in mash form and tap water were available for ad libitum consumption. Lighting was provided for 24 hr daily. Care and management of the birds followed recommended guidelines (FASS, 2010). The University of Arkansas Institutional Animal Use and Care Committee approved all procedures.

Measurements

Body weight by pen was taken at 35 and 49 d of age with feed consumption during the experimental period determined. Birds were checked twice daily and any bird that died or was removed to alleviate suffering was weighed with the weight used to adjust feed conversion. At the conclusion of the study two birds per pen were processed in a pilot plant as described by Fritts and Waldroup (2006) to determine dressing percentage and parts yield.

Statistical Analysis

Pen means served as the experimental unit for statistical analysis. Data were subjected to ANOVA as a factorial arrangement of treatments with dietary Lys level and supplemental Met level as the main effects with the interaction of dietary Lys and supplemental Met level using the General Linear Models procedure of SAS (SAS Institute, 1991). When significant differences among treatments were found, means were separated using repeated t-test using the LSMEANS option of the GLM procedure. Mortality data were transformed to $\sqrt{n+1}$ before analysis; Data were presented as natural numbers. Significant statements are based on p≤ 0.05. Nonlinear regression analysis was conducted using the PROC LIN procedure of SAS (SAS Institute, 1991) and the SAS macro of Robbins (1986) to determine the level of Met, TSAA, and ratios of Met: Lys and TSAA: Lys that provide the greatest response in FI, BWG, and FCR at each level of dietary lysine.

RESULTS AND DISCUSSION

Analyzed crude protein and amino acid contents in the basal diets were in close agreement with the calculated values. The analyzed free supplemental amino acids fell within the calculated values.

Table 5 shows the effects of various dLys levels and supplemental Met levels on FI, BWG and FCR. There was a significant effect of dLys level on BW and FCR (P≤0.05). As the dLys level increased from 0.6 to 0.9%, the BWG increased significantly. There was no improvement of BWG with further increase of dLys. FCR was reduced significantly as dLys level increased from 0.6 to 1.0% but no further reduction with further increase of dLys level. There was no significant effect of dLys levels on FI (P≤0.05). There was a significant effect of the supplemental Met on FCR but not on FI and BWG (P≤0.05). Further supplementation of Met with 0.2 and 0.4% did not improve FCR. The digestible Met (dMet) level in basal diets with 0.6%dLys is 0.18%. Based on an estimated 88% digestibility of the amino acids in a typical soybean meal diet (Heartland Lysine, 1995), the total Met level in basal diets would be

0.20%, which is lower than the NRC (1994) recommended level. Therefore, the supplementation of Met to the basal diets improved FCR. Once the supplementation of Met increased to 0.2%, there was no further improvement of FCR, which indicated that the Met level in the diets was sufficient to support FCR. Vieira et al. (2004) conducted a similar study using 4 graded levels of Met with a fixed Lys level (1.12 or 1.46%) in the diets for two strains of broilers from 14 to 35 d of age. They reported that increasing Met levels resulted in significant effect (nonlinear or linear) on BWG and FCR but not on FI for both strains.

Broken-line regression analysis results (Table 6) showed that the estimate of dMet level for optimal FI at 0.6% Lys level is 0.460±0.122. For optimal BWG, the estimate of dMet level at dLys levels of 0.6 and 0.7% is0.297±0.594 and 0.481±0.222. For optimal FCR, the estimate of dMet level at dLys levels of 0.6 % is 0.355±0.215.The estimates of dMet levels for optimal FI, BWG, and FCR at the rest of tested Lys levels during this period could not be converged. As the Lys level in the basal diet increased, the basal Met level also increased. Therefore the first one or two Met supplementation to the basal diets is probably sufficient to support the performance and this is also why there was no convergence in the regression analysis for the rest of supplementation.

Broken-line regression analysis results (Table 7) showed that the estimate of digestible TSAA (dTSAA) level for optimal FI at 0.6% Lys level is 0.640±0.122. For optimal BWG, the estimate of dTSAA level at dLys levels of 0.6 and 0.7% is 0.477±0.594 and 0.681±0.222. For optimal FCR, the estimate of dTSAA level at dLys levels of 0.6 % is 0.535±0.215. The estimates of dTSAA levels for optimal FI, BWG, and FCR at the rest of tested Lys levels during this period could not be converged.

Regression analysis was used to evaluate the ratio of Met to Lys for optimal performance as each level of Lys increased from 0.6 to 1.1% (Table 8). The ratio of Met to Lys for optimal FI at dLys levels of 0.6 is 76.6±20.2. For optimal BWG, the ratio of Met to Lys at dLys levels of 0.6 and 0.7% is 49.5±99.2 and 68.7±31.7, respectively. It shows that optimal ratio of Met to Lys increased as the Lys level increased from 0.6 to 0.7%. For optimal FCR, the estimated ratio of Met to Lys at dLys levels of 0.6 is 59.2±36.0. The estimated ratios of Met to Lys for optimal FI, BWG, and FCR at the rest of tested dLys levels could not be converged. As aforementioned above, this is probably due to the sufficient supplementation of Met on the first or second supplemented diets.

The ratios of TSAA to Lys were also estimated with broken-line regression (Table 9). The results showed that the estimated ratio of TSAA to Lys for optimal FI at dLys levels of 0.6% is 106.6±20.2. For optimal BWG, the ratio of TSAA to Lys at dLys levels of 0.6 and 0.7 % is 79.5±99.2, 97.3±31.7, respectively. The ratios of TSAA to Lys for optimal BWG increased as the dLys level increased from 0.6 to 0.7%. For optimal FCR, the estimate of TSAA to Lys ratio at dLys levels of 0.6% is 89.2±36.0. These ratios were higher than the one reported by Mack et al. (1999). They estimated that when the digestible Lys required for BWG was 0.86% in the diet, the ideal ratio of TSAA to Lys was 75. The estimated ratios of TSAA to Lys for FI, BWG, and FCR at the rest of tested dLys levels could not be converged.

Regardless of Met variation in the diets, regression analysis showed (Table 10) that the optimal dLys requirement for FI, BWG, and FCR during this period is 0.986±0.013, 1.013±0.056, and 1.052±0.020, respectively. The requirement of dLys for FCR is slightly higher than the level for BWG. This response was similar to that of the studies from Baker *et al.* (2002) and Garcia et al. (2006), who showed that the dLys requirement for optimal FCR was higher than that for maximal BWG for broiler chickens.

No significant interactions were observed between Lys and supplemental Met for FI, BWG, and FCR ($P>0.05$). Similarly, Si *et al.* (2004) reported that when both Met and Lys were fed equal to or in excess of NRC recommendations, there were no significant interactions between Lys and Met for BWG, FCR, or breast meat yield. No significant effect of Lys or supplemental Met levels on mortality was observed (data not shown). During the experimental period of 35 to 49 d, the total mortality rate was 2.64%.

The effects of various dLys levels and supplemental Met levels on processing characteristics at 49d are shown in Table 11, 12, and 13. There were no significant interactions between dLys and supplemental Met on dressing percentage. Increasing dLys level significantly improved dressing percentage. There was no significant effect of supplemental Met on dressing percentage (Table 11). Based on percentage of live weight, there were no significant interactions between dLys and supplemental Met on parts yield. Increasing dLys level significantly increased breast and leg quarter yield (Table 12). However, there was no significant effect of dLys level on leg quarter yield based on percentage of carcass weight (Table 13). Supplemental Met had no significant effects on any of the processing parameter measured.

The results of this study showed that the optimal ratios of Met or TSAA to Lys for each of these parameters (FI, BWG, and FCR) vary with diets using the same or different dLys levels. Therefore, when formulating a diet using Ideal Protein Concept, it is important to know the ideal amino acid profile at a specific dietary Lys level used in the diet.

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Table1 Recommendations for amino acid ratios to lysine by various researchers

*AA	**1						2	3	4	5	6	7	8	9	10		All Ages		
	1-21		22-42		43-56			1-21	20-40	7-28	8-21	0-21	0-42	1-21	10-21	32-43			
	Dig	Tot	Dig	Tot	Dig	Tot	Dig	Dig	Dig	Dig	Dig	Dig	Dig	Tot	Dig	Dig	Mean	Low	High
Lys	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Arg	105	102	105	102	105	102	110	105	112	108		96	105	105	97	101	104	96	112
Gly								65									65		
Gly+Ser		150		140		135								150			144	135	150
His	36	36	36	36	36	36		32		38		24		29	29	31	33	24	38
Ile	65	66	67	68	67	68	70	67	71	63	61.4	65	66	67	64	62	66	61.4	71
Leu	108	108	109	109	109	109		109		108		92		100	94	117	106	92	117
Met	39	39	40	40	40	40				37		38	38	42	35	36	39	35	42
TSAA	71	71	72	72	72	72	75	72	75	70		72	73	75	71	69	72	69	75
Phe	63	63	63	63	63	63				62				60			63	60	63
Phe+Tyr	115	114	115	114	115	114		105		121				112	107	102	112	102	121
Pro								44									44		
Thr	65	68	65	68	65	68	65	67	63	66	55.7	62	65	67	63	65	65	62	68
Trp	16	16	17	17	17	17	18	16	19	14	16.6	18	16	17	16	18	17	14	19
Val	75	76	77	78	77	78	80	77	81	81	77.5	69	80	75	67	75	76	67	81

*AA-amino acid; Dig-digestible; Tot-total

**1. Rostagno et al., 2005; 2. Schutte and de Jong, 1999; 3. Baker and Han, 1994; 4.Mack et al., 1999; 5.Roth et al., 2001; 6. Baker et al., 2002; 7. Austic, 1994; 8. Dutch Bureau of Livestock Feeding, 1996. 9. NRC 1994 with Lys adjusted to 1.20% and Gly+Ser at 1.80%; 10. Coon, 2004.

Table2 Composition (g/kg) of diets with variable levels of lysine using composite Ideal Ratio

Ingredient	% Digestible Lysine					
	0.60	0.70	0.80	0.90	1.00	1.10
Yellow corn	830.85	788.67	746.22	693.74	641.26	588.78
Soybean meal	121.40	158.31	195.45	241.73	288.00	334.29
Poultry oil	8.09	13.63	19.20	26.21	33.23	40.24
Limestone	12.93	12.58	12.23	11.79	11.35	10.91
Dicalcium phosphate	10.87	10.64	10.42	10.14	9.86	9.58
Sodium chloride	4.00	4.00	4.00	4.00	4.00	4.00
L-Lysine HCl	1.78	1.94	2.10	1.98	1.87	1.75
L-Threonine	0.08	0.23	0.38	0.41	0.43	0.45
DL methionine	0.00	0.00	0.00	0.00	0.00	0.00
Vitamin premix[1]	5.00	5.00	5.00	5.00	5.00	5.00
Trace mineral[2]	1.00	1.00	1.00	1.00	1.00	1.00
Variable[3]	4.00	4.00	4.00	4.00	4.00	4.00
TOTAL	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00

[1]Provides per kg of diet: vitamin A (from vitamin A acetate) 7715 IU; cholecalciferol 5511 IU; vitamin E (from dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadionedimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin (from thiamin mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg

[2] Provides per kg of diet: Mn (from MnSO4·H20) 100 mg; Zn (from ZnSO4·7H2O) 100 mg; Fe (from FeSO4·7H 2O) 50 mg; Cu (from CuSO4·5H20) 10 mg; I from Ca(IO3)2·H20), 1.0 mg.

[3]Variable amounts of DL methionine and washed builders sand.

Table 3 Calculated nutrient content of test diets[1] (Values in bold italic are at minimum specified level).

Nutrient (%)	% Digestible Lysine						NRC[2]
	0.60	0.70	0.80	0.90	1.00	1.10	
Crude protein	12.10	13.53	14.98	16.76	18.49	20.25	18.00
Met	0.20	0.22	0.23	0.26	0.28	0.30	0.32
Lys	0.68	0.79	0.90	1.01	1.12	1.24	0.85
Trp	0.13	0.15	0.17	0.20	0.23	0.25	0.16
Thr	0.46	0.54	0.60	0.68	0.75	0.83	0.68
Ile	0.47	0.54	0.60	0.69	0.77	0.86	0.62
His	0.33	0.37	0.41	0.46	0.50	0.55	0.27
Val	0.56	0.63	0.69	0.78	0.86	0.95	0.70
Leu	1.18	1.27	1.37	1.48	1.60	1.72	0.93
Arg	0.70	0.81	0.92	1.07	1.21	1.35	1.00
TSAA	0.43	0.47	0.50	0.55	0.59	0.63	0.60
Phe+Tyr	1.00	1.13	1.25	1.41	1.57	1.73	1.04
Gly+Ser	1.04	1.18	1.31	1.48	1.65	1.83	0.97
dMet	0.18	0.19	0.21	0.23	0.25	0.27	----
dLys	***0.60***	***0.70***	***0.80***	***0.90***	***1.00***	***1.10***	----
dTrp	0.11	0.13	0.14	0.17	0.19	0.21	----
dThr	***0.39***	***0.46***	***0.52***	***0.59***	***0.65***	***0.72***	----
dIle	0.41	0.47	0.53	0.61	0.68	0.76	----
dHis	0.29	0.32	0.36	0.40	0.44	0.48	----
dVal	0.47	0.54	***0.61***	***0.68***	***0.76***	***0.84***	----
dArg	***0.62***	***0.73***	0.83	0.96	1.09	1.22	----
dTSAA	0.36	0.39	0.43	0.46	0.50	0.54	----
dPhe+tyr	0.93	1.05	1.17	1.32	1.46	1.61	----

[1] All diets calculated to contain 3100 ME kcal/kg, 0.80% Ca, and 0.30% Nonphytate Phosphorus
[2]NRC (1994) suggested requirements for 6 to 8 wk.

Table 4 Met: Lys and TSAA: Lys ratios at each level of Lys

Trt	Dig Lys %	Basal Dig Met %	Added Met activity %	Dig Met %	Basal Dig TSAA %	Dig TSAA **%**	Met:Lys ratio	TSAA:Lys ratio
1	0.6	0.18	0.0	0.18	0.36	0.36	30.0	60.0
2	0.6	0.18	0.1	0.28	0.36	0.46	46.7	76.7
3	0.6	0.18	0.2	0.38	0.36	0.56	63.3	93.3
4	0.6	0.18	0.4	0.58	0.36	0.76	96.7	126.7
5	0.7	0.19	0.0	0.19	0.39	0.39	27.1	55.7
6	0.7	0.19	0.1	0.29	0.39	0.49	41.4	70.0
7	0.7	0.19	0.2	0.39	0.39	0.59	55.7	84.3
8	0.7	0.19	0.4	0.59	0.39	0.79	84.3	112.9
9	0.8	0.21	0.0	0.21	0.43	0.43	26.3	53.8
10	0.8	0.21	0.1	0.31	0.43	0.53	38.8	66.3
11	0.8	0.21	0.2	0.41	0.43	0.63	51.3	78.8
12	0.8	0.21	0.4	0.61	0.43	0.83	76.3	103.8
13	0.9	0.23	0.0	0.23	0.46	0.46	25.6	51.1
14	0.9	0.23	0.1	0.33	0.46	0.56	36.7	62.2
15	0.9	0.23	0.2	0.43	0.46	0.66	47.8	73.3
16	0.9	0.23	0.4	0.63	0.46	0.86	70.0	95.6
17	1.0	0.25	0.0	0.25	0.50	0.50	25.0	50.0
18	1.0	0.25	0.1	0.35	0.50	0.60	35.0	60.0
19	1.0	0.25	0.2	0.45	0.50	0.70	45.0	70.0
20	1.0	0.25	0.4	0.65	0.50	0.90	65.0	90.0
21	1.1	0.27	0.0	0.27	0.54	0.54	24.5	49.1
22	1.1	0.27	0.1	0.37	0.54	0.64	33.6	58.2
23	1.1	0.27	0.2	0.47	0.54	0.74	42.7	67.3
24	1.1	0.27	0.4	0.67	0.54	0.94	60.9	85.5

Table 5 Effect of dLys and supplemental Met on live performance of 35-49d-old broilers

Added Met,%	Dig Lys, %						Mean
	0.6	0.7	0.8	0.9	1.0	1.1	
Feed intake							
0.0	2.791	2.628	2.586	2.602	2.632	2.643	2.647
0.1	2.662	2.630	2.600	2.623	2.601	2.510	2.604
0.2	2.653	2.640	2.753	2.600	2.681	2.389	2.619
0.4	2.578	2.742	2.584	2.684	2.536	2.571	2.616
Mean	2.671	2.660	2.631	2.627	2.612	2.528	
Body weight gain(kg)							
0.0	0.960	1.022	1.021	1.141	1.184	1.222	1.092
0.1	0.973	1.149	1.075	1.169	1.288	1.231	1.147
0.2	1.001	1.054	1.159	1.198	1.283	1.191	1.148
0.4	0.965	1.098	1.083	1.207	1.224	1.326	1.151
Mean	0.975^{c}	1.081^{b}	1.085^{b}	1.179^{a}	1.245^{a}	1.242^{a}	
Feed conversion ratio(kg: kg)							
0.0	2.813	2.694	2.513	2.282	2.236	2.172	2.452^{x}
0.1	2.745	2.432	2.429	2.266	2.024	1.953	2.308^{y}
0.2	2.643	2.510	2.318	2.174	2.104	2.050	2.300^{y}
0.4	2.745	2.510	2.342	2.227	2.090	1.885	2.300^{y}
Mean	2.737^{a}	2.537^{b}	2.401^{c}	2.237^{d}	2.113^{e}	2.015^{e}	

	Feed intake		Weight gain		FCR	
	P-value	SEM	P-value	SEM	P-value	SEM
Lys	0.059	0.041	<0.0001	0.032	<0.0001	0.046
Added Met	0.742	0.029	0.249	0.027	0.004	0.038
Lys x Added Met	0.148	0.090	0.931	0.082	0.940	0.115

^{abcde} means in rows with common superscripts do not differ significantly($p \leq 0.05$).

^{xy} means in columns with common superscripts do not differ significantly($p \leq 0.05$).

Table 6 Estimates of digestible Met requirement at different Lys levels

Variable	parameter	Met Estimate	SE	CI	
				Low	High
FI 35-49d	Lys 0.6	0.460	0.122	0.000	2.009
	Lys 0.7	Non convergence			
	Lys 0.8	Non convergence			
	Lys 0.9	Non convergence			
	Lys 1.0	Non convergence			
	Lys 1.1	Non convergence			
BWG 35-49d	Lys 0.6	0.297	0.594	0.000	7.843
	Lys 0.7	0.481	0.222	0.000	3.297
	Lys 0.8	Non convergence			
	Lys 0.9	Non convergence			
	Lys 1.0	Non convergence			
	Lys 1.1	Non convergence			
FCR 35-49d	Lys 0.6	0.355	0.215	0.000	3.092
	Lys 0.7	Non convergence			
	Lys 0.8	Non convergence			
	Lys 0.9	Non convergence			
	Lys 1.0	Non convergence			
	Lys 1.1	Non convergence			

Table 7 Estimates of digestible TSAA requirement at different Lys levels

Variable	parameter	TSAA Estimate	SE	CI	
				Low	High
FI 35-49d	Lys 0.6	0.640	0.122	0.000	2.189
	Lys 0.7	Non convergence			
	Lys 0.8	Non convergence			
	Lys 0.9	Non convergence			
	Lys 1.0	Non convergence			
	Lys 1.1	Non convergence			
BWG 35-49d	Lys 0.6	0.477	0.594	0.000	8.023
	Lys 0.7	0.681	0.222	0.000	3.497
	Lys 0.8	Non convergence			
	Lys 0.9	Non convergence			
	Lys 1.0	Non convergence			
	Lys 1.1	Non convergence			
FCR 35-49d	Lys 0.6	0.535	0.215	0.000	3.272
	Lys 0.7	Non convergence			
	Lys 0.8	Non convergence			
	Lys 0.9	Non convergence			
	Lys 1.0	Non convergence			
	Lys 1.1	Non convergence			

Table 8 Estimates of Met/Lys ratio at different Lys levels

Variable	parameter	Met/Lys Estimate	SE	CI	
				Low	High
FI 35-49d	Lys 0.6	76.6	20.2	0.0	333.0
	Lys 0.7	Non convergence			
	Lys 0.8	Non convergence			
	Lys 0.9	Non convergence			
	Lys 1.0	Non convergence			
	Lys 1.1	Non convergence			
BWG 35-49d	Lys 0.6	49.5	99.2	0.0	1309.0
	Lys 0.7	68.7	31.7	0.0	471.4
	Lys 0.8	Non convergence			
	Lys 0.9	Non convergence			
	Lys 1.0	Non convergence			
	Lys 1.1	Non convergence			
FCR 35-49d	Lys 0.6	59.2	36.0	0.0	516.3
	Lys 0.7	Non convergence			
	Lys 0.8	Non convergence			
	Lys 0.9	Non convergence			
	Lys 1.0	Non convergence			
	Lys 1.1	Non convergence			

Table 9 Estimates of TSAA/Lys ratio at different Lys levels

Variable	parameter	TSAA/Lys Estimate	SE	CI	
				Low	High
FI 35-49d	Lys 0.6	106.6	20.2	0.0	363.2
	Lys 0.7	Non convergence			
	Lys 0.8	Non convergence			
	Lys 0.9	Non convergence			
	Lys 1.0	Non convergence			
	Lys 1.1	Non convergence			
BWG 35-49d	Lys 0.6	79.5	99.2	0.0	1339.7
	Lys 0.7	97.3	31.7	0.0	500.0
	Lys 0.8	Non convergence			
	Lys 0.9	Non convergence			
	Lys 1.0	Non convergence			
	Lys 1.1	Non convergence			
FCR 35-49d	Lys 0.6	89.2	36.0	0.0	546.3
	Lys 0.7	Non convergence			
	Lys 0.8	Non convergence			
	Lys 0.9	Non convergence			
	Lys 1.0	Non convergence			
	Lys 1.1	Non convergence			

Table 10 Estimates of Lys levels at all Met levels for FI, BWG, and FCR

Period	Criteria	Lys Estimate	SE	CI	
				Low	High
35-49 d	FI	0.986	0.013	0.930	1.041
	BWG	1.013	0.056	0.836	1.189
	FCR	1.052	0.020	0.989	1.115

Table 11 Effect of Lys and added Met on dressing percentage of 49d-old broilers

Added Met,%	Dig Lys, %						Mean
	0.6	0.7	0.8	0.9	1.0	1.1	
Dressing percentage[1] (%)							
0.0	72.33	72.30	72.72	73.50	72.08	73.72	72.77
0.1	72.76	72.44	72.71	73.06	73.57	74.24	73.12
0.2	72.78	73.20	72.61	72.98	73.74	73.49	73.13
0.4	72.75	72.85	73.22	73.10	73.90	73.36	73.20
Mean	72.66[c]	72.70[bc]	72.81[bc]	73.16[abc]	73.32[ab]	73.70[a]	

	Dressing Percentage	
	P-value	SEM
Lys	0.010	0.242
AdMet	0.399	0.194
Lys x AdMet	0.583	0.521

[1] hot eviscerated carcass as a percentage of live weight

Table 12 Effect of Lys and added Met on parts yield (as % of live weight) of 49d-old broilers

AdMet,%	Dig Lys, %						Mean
	0.6	0.7	0.8	0.9	1.0	1.1	
Breast yield[1] (%)							
0.0	21.52	21.29	22.10	22.39	21.94	22.88	22.02
0.1	21.72	21.84	21.56	23.02	22.90	24.15	22.53
0.2	21.32	21.77	22.36	22.68	22.96	23.31	22.40
0.4	21.83	22.30	22.44	23.15	22.33	22.50	22.43
Mean	21.59[d]	21.80[cd]	22.12[bcd]	22.81[ab]	22.53[abc]	23.21[a]	
Leg quarter yield (%)							
0.0	22.40	23.17	22.32	23.25	22.97	23.46	22.93
0.1	22.45	22.82	22.40	22.80	23.36	23.22	22.84
0.2	23.01	22.58	22.77	22.99	22.38	23.25	22.83
0.4	22.87	22.37	22.52	22.63	24.15	23.66	23.03
Mean	22.68[c]	22.73[c]	22.50[c]	22.91[bc]	23.22[ab]	23.40[a]	
Wings yield (%)							
0.0	7.82	7.89	7.76	7.90	7.63	7.64	7.78
0.1	7.86	7.64	8.01	7.54	7.72	7.65	7.74
0.2	7.77	7.81	7.61	7.61	7.60	7.61	7.67
0.4	7.56	7.75	7.86	7.88	7.62	7.92	7.76
Mean	7.75	7.77	7.81	7.73	7.64	7.70	
		Breast yield		Leg quarter yield		Wings yield	
		P-value	SEM	P-value	SEM	P-value	SEM
Lys		0.0001	0.279	0.0009	0.171	0.566	0.067
AdMet		0.386	0.223	0.696	0.137	0.465	0.054
Lys x AdMet		0.810	0.599	0.080	0.367	0.266	0.145

[1] skinless, boneless breast meat (pectoralis major+pectoralis minor) as a percentage of live weight

Table 13 Effect of Lys and added Met on parts yield (as % of carcass weight) of 49d-old broilers

AdMet,%	Dig Lys, %						Mean
	0.6	0.7	0.8	0.9	1.0	1.1	
Breast yield[1] (%)							
0.0	29.72	29.42	30.34	30.45	30.42	31.02	30.23
0.1	29.84	30.11	29.64	31.50	31.12	32.52	30.79
0.2	29.28	29.74	30.79	31.03	31.13	31.72	30.61
0.4	30.00	30.60	30.60	31.64	30.16	30.66	30.61
Mean	29.71^{d}	29.97^{cd}	30.34^{bcd}	31.15^{ab}	30.71^{abc}	31.48^{a}	
Leg quarter yield (%)							
0.0	30.98	32.04	30.70	31.62	31.88	31.81	31.51
0.1	30.85	31.51	30.82	31.21	31.78	31.28	31.24
0.2	31.61	30.85	31.38	31.52	30.35	31.64	31.23
0.4	31.42	30.72	30.78	30.98	32.71	32.25	31.48
Mean	31.22	31.28	30.92	31.33	31.68	31.75	
Wings yield (%)							
0.0	10.81	10.92	10.68	10.76	10.59	10.36	10.69
0.1	10.80	10.55	11.02	10.32	10.50	10.31	10.58
0.2	10.69	10.67	10.48	10.43	10.30	10.35	10.49
0.4	10.39	10.64	10.74	10.78	10.33	10.80	10.61
Mean	10.67	10.69	10.73	10.57	10.43	10.46	

	Breast yield		Leg quarter yield		Wings yield	
	P-value	SEM	P-value	SEM	P-value	SEM
Lys	0.0003	0.322	0.111	0.238	0.089	0.095
AdMet	0.472	0.258	0.593	0.190	0.287	0.076
Lys x AdMet	0.699	0.692	0.065	0.510	0.277	0.203

[1] skinless, boneless breast meat (pectoralis major+pectoralis minor) as a percentage of carcass weight

Overall Conclusion

The results of experiment 1 indicated that optimal ratios of Met or TSAA to Lys may depend on dietary Lys level in broiler starter diet. Experiment 2 showed that optimal ratio of Met or TSAA to Lys varies with different dietary Lys level in grower diets. Similarly, the ideal amino acid profile for Met or TSAA relative to Lys may depend on Lys level in broiler finisher diets. In conclusion, the ideal ratios of Met or TSAA to Lys based on the Ideal Protein Concept vary for broiler chickens fed different Lys level in the diets at each of starter, grower, and finisher phases.